Conseil Supérieur de la Santé

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Évaluation des effets des néonicotinoïdes et du fipronil sur la biodiversité et la santé

Dans le présent rapport scientifique sur les politiques de santé publique, le Conseil Supérieur de la Santé de Belgique propose une analyse de l'Évaluation Mondiale Intégrée (WIA) de l'impact des néonicotinoïdes et du fipronil sur la biodiversité et les écosystèmes et place les conclusions de cette évaluation et d'une étude similaire plus récente, réalisée par l'EASAC, dans le contexte élargi des politiques européenne et belge sur les pesticides et du rôle positif des services écosystémiques dans la santé humaine.

Le Conseil Supérieur de la Santé est parvenu à la conclusion que les résultats de la WIA et de l'étude de l'EASAC relatives aux effets sur la santé des personnes et des écosystèmes constituent de sérieux avertissements et qu'il est urgent de réaliser une évaluation plus approfondie de la toxicité de ces composés, de leur action sur l'organisme humain et de leurs effets sur les services écosystémiques.

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RÉSUMÉ

Les insecticides néonicotinoïdes contiennent cinq substances actives qui, de même que l'insecticide fipronil, sont principalement et largement utilisées dans la production végétale à l'échelle mondiale et en Belgique. L'utilisation de ces pesticides a récemment été associée au déclin des colonies d'abeilles et d'autres populations de pollinisateurs. En 2014, un comité scientifique international, soutenu par l'Union internationale pour la conservation de la nature, a publié une analyse approfondie des effets de ces pesticides sur les insectes pollinisateurs, d'autres espèces et les écosystèmes intitulée l'« Évaluation Mondiale Intégrée de l'impact des pesticides systémiques sur la biodiversité et les écosystèmes » (WIA, acronyme de *Worldwide Integrated Assessment*). Cette étude concluait qu'il y a lieu de se préoccuper des effets négatifs des substances en question sur les insectes pollinisateurs, d'autres espèces et le fonctionnement des écosystèmes.

[1] Le Conseil se réserve le droit d'apporter à tout moment des corrections typographiques mineures au présent document. Les changements qui en modifient le contenu sont en revanche automatiquement inclus dans un erratum. Dans ce cas, une nouvelle version du rapport consultatif est publiée.

La publication de la WIA a conduit les ministres fédéraux de la Santé publique et de l'Agriculture, ainsi que l'ancien secrétaire d'État à l'Environnement, à demander au Conseil Supérieur de la Santé (CSS) de se pencher sur cette étude, en s'intéressant plus particulièrement à sa qualité scientifique et à l'applicabilité de ses conclusions à la Belgique. Le présent rapport répond à cette demande gouvernementale et place les résultats de la WIA ainsi que les conclusions d'une étude similaire, plus récente (2015), de *l'European Academies Science Advisory Council* (EASAC) et celles de Godfray et coll. (2014, 2015) dans le contexte élargi des politiques européenne et belge sur les pesticides et du rôle positif des services écosystémiques dans la santé humaine.

WIA

La WIA n'est ni une revue systématique ni une méta-analyse. Elle doit être considérée comme une « évaluation approfondie » similaire aux rapports d'autres organisations nationales et internationales telles que le Conseil Supérieur de la Santé (CSS). D'après les conclusions de ses auteurs, les données examinées démontrent que les effets sur les insectes pollinisateurs, d'autres espèces et les écosystèmes nuisent probablement à l'environnement. La WIA (2014) propose une synthèse de 1121 études publiées dans des revues à comité de lecture portant principalement sur les cinq dernières années, et inclut également les études financées par l'industrie. Bien que l'étude gagnerait en clarté si elle fournissait davantage d'informations sur la stratégie de recherche bibliographique et de sélection, rien ne permet au CSS de déduire que cette stratégie manque d'objectivité. Le Conseil est donc parvenu à la conclusion – renforcée par la concordance des résultats de la WIA avec ceux de l'étude de l'EASAC – que ses résultats doivent être considérés avec sérieux. S'il est vrai que la WIA ne tient pas compte de tous les éléments nécessaires à la réalisation d'une évaluation complète de l'impact sur la santé humaine et environnementale et que les concentrations dans l'environnement varient d'une région à l'autre en fonction des pratiques agricoles, le CSS juge néanmoins pertinentes pour la Belgique les préoccupations soulevées dans la WIA et l'étude de l'EASAC.

Santé humaine

Il a été observé qu'outre leur incidence sur la santé des écosystèmes, les insecticides dont il est question dans le présent avis sont neurotoxiques. Le thiaclopride a été reconnu comme étant cancérigène. Des effets génotoxiques ont été observés in vitro, dans des cellules humaines et à l'aide de tests in vivo sur des animaux, mais sont plus difficiles à démontrer par une recherche épidémiologique. Un nombre croissant d'éléments souligne, entre autres impacts, des effets perturbant le système endocrinien à des niveaux d'exposition de plus en plus bas. Le fipronil est suspecté de perturber le fonctionnement du système endocrinien. La vie fœtale et la prime enfance s'avèrent être des périodes d'exposition critiques.

Les niveaux de référence européens concernant l'exposition professionnelle à l'acétamipride et à l'imidaclopride, qui appartiennent tous deux à la famille des néonicotinoïdes, ont été réduits en 2013, et la toxicité des autres composés sera réévaluée au cours des années à venir. Les effets des expositions chroniques (par la chaîne alimentaire par ex.) sont incertains,

de même que les effets d'une exposition cumulée à des mélanges de ces composés entre eux ou avec d'autres polluants.
En dehors des effets d'une exposition directe à ces composés, une préoccupation croissante (également étayée par des publications plus récentes que les rapports de la WIA et de l'EASAC) concerne l'impact sur la santé humaine du déclin de la qualité des aliments et de la production en raison de leurs effets sur les insectes pollinisateurs et les services écosystémiques. Malgré l'incertitude qui entoure l'effet dans le temps et la gravité de ces composés, le CSS recommande l'adoption d'une approche préventive, notamment en Belgique. Il est peu probable que les concentrations actuelles dans l'environnement aient de graves effets sur la santé, mais le Conseil réitère sa préoccupation concernant les effets des expositions chroniques découlant de l'utilisation massive de ces pesticides.

Lutte contre les ennemis des cultures

Un moyen innovant d'employer ces insecticides consiste à les utiliser comme enrobage de semences, ce qui présente des avantages pour la pratique agricole et réduit l'exposition des agriculteurs et des personnes vivant dans les zones d'épandage des pesticides. Les produits phytopharmaceutiques doivent être utilisés de façon à laisser la plus petite quantité de résidus possible.

D'après les politiques européenne et belge en matière de lutte intégrée contre les ennemis des cultures, les méthodes chimiques de lutte contre les ennemis des cultures doivent être utilisées en dernier recours. La lutte intégrée peut être considérée dans le cadre d'une stratégie préventive pour répondre aux préoccupations relatives aux effets des pesticides sur la santé des personnes et des écosystèmes, même si les données scientifiques comportent un degré élevé d'incertitudes.

Recherche

Compte tenu des lacunes existantes, de l'utilisation massive des insecticides en question, du développement d'une résistance aux pesticides – qui conduit à l'utilisation de substances encore plus puissantes – et des préoccupations concernant les effets des expositions chroniques sur la santé publique, le CSS recommande vivement de poursuivre les recherches scientifiques sur les propriétés des composés (ainsi que des produits similaires) et leurs effets sur la santé des personnes et des écosystèmes. Les efforts de recherche doivent, de préférence, être coordonnés à l'échelle européenne, voire internationale. En dehors de la réalisation de telles études, le CSS recommande de redoubler d'efforts pour concevoir d'autres méthodes et outils de lutte contre les ennemis des cultures. La lutte intégrée, de même que la poursuite des recherches, exige l'intervention d'un groupe interdisciplinaire d'experts compétents et de divers acteurs sociaux concernés afin d'évaluer les conséquences sociales (sur l'économie, la santé, etc.) des différentes options de lutte.

Conclusion

En résumé, le CSS conclut que les résultats de la WIA et de l'étude de l'EASAC relatives aux effets sur la santé des personnes et des écosystèmes constituent d'importants avertissements (préliminaires). Ces résultats incitent le Conseil à prôner le renforcement de la transition vers des pratiques de lutte intégrée pour réduire l'utilisation des néonicotinoïdes et du fipronil.
Il est par ailleurs urgent de réaliser une nouvelle évaluation de la toxicité de ces composés, de leur action sur l'organisme humain et de leurs effets sur les services écosystémiques, dus, par exemple, au déclin de la population des insectes pollinisateurs. Enfin, la participation des parties prenantes est fortement recommandée afin d'évaluer l'impact social des différentes options de lutte.

Keywords and MeSH *descriptor terms*[2]

MeSH terms*	**Keywords**	**Sleutelwoorden**	**Mots clés**	**Schlüsselwörter**
-	neonicotinoids	neonicotinoïden	néonicotinoïdes	Neonicotinoide
“fipronil”	fipronil	fipronil	fipronil	Fipronil
“biodiversity”	biodiversity	biodiversiteit	biodiversité	Biodiversität
“health”	health	gezondheid	santé	Gesundheit
“Belgium”	Belgium	België	Belgique	Belgien
“government regulation”	regulation	regelgeving	réglementation	Regulierung
-	methods assessment	methodenbeoordeling	Évaluation des méthodes	Methodenbewertung

MeSH (Medical Subject Headings) is the NLM (National Library of Medicine) controlled vocabulary thesaurus used for indexing articles for PubMed http://www.ncbi.nlm.nih.gov/mesh.

[2] The Council wishes to clarify that the MeSH terms and keywords are used for referencing purposes as well as to provide an easy definition of the scope of the advisory report. For more information, see the section entitled "methodology".

CONTENTS

ABBREVIATIONS AND SYMBOLS

AChE	Acetylcholinesterase
ADHD	Attention Deficit Hyperactivity Disorder
ADI	Acceptable Daily Intake
AKP	Alkaline Phosphatase
AOEL	Acceptable Operator Exposure Level
aOR	adjusted Odds Ratio
ARfD	Acute Reference Dose
BW	Body Weight
CI	Confidence Interval
CK	Creatinine Kinase
CNS	Central Nervous System
DAR	Draft Assessment Report
DNA	Deoxyribonucleic Acid
DNT	Developmental Neurotoxicity
DT_{50}	degradation time or the time that has to pass to degrade 50 % of the initial dose
EASAC	European Academies Science Advisory Council
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
EU	European Union
FASFC	Federal Agency for the Safety of the Food Chain
FOB	Functional Observational Battery
FPS	Federal Public Service
GABA	γ-aminobutyric acid
GD	Gestation Day
GFAP	Glial Fibrillary Acidic Protein
IPM	Integrated Pest Management
IQR	Interquartile Range
IWT	*Agentschap voor Innovatie door Wetenschap en Technologie* / Agency for Innovation by Science and Technology
Koc	Soil Organic Carbon-Water Partitioning Coefficient
Kow	Octanol-Water Partitioning Coefficient
LD	Lactation Day
LD50	Median Lethal Dose
LDH	Lactate Dehydrogenase
LOAEL	Lowest Observed Adverse Effect Level
LPO	Lipid Peroxidation
MAC	Maximum Allowable Concentration
nAChR	nicotinic Acetylcholine Receptor
NOAEL	No Observed Adverse Effect Level
NT	Neurotoxic
PND	Postnatal Day
PPP	Plant Protection Product
PPR	EFSA scientific Panel on Plant Protection Products and their Residues

Pv	vapour Pressure
RfD	Reference Doses
S	water solubility
SENSOR	Sentinel Event Notification System for Occupational Risks
SHC	Superior Health Council
SLA	Spontaneous Locomotor Activity
TRH	Thyrotropin Releasing Hormone
TSH	Thyroid Stimulating Hormone
vet subst	veterinarian substance
VITO	*Vlaamse Instelling voor Technologisch Onderzoek*
WIA	Worldwide Integrated Assessment on the risks of neonicotinoids and fipronil to biodiversity and ecosystem functioning

I. INTRODUCTION ET QUESTIONS

Depuis 1994, les apiculteurs français signalent des changements alarmants dans le comportement des abeilles à miel (*Apis mellifera*) : bon nombre d'entre elles ne retournent pas à leur ruche, elles perdent leur orientation, manifestent un comportement de butinage anormal et leur population décline en raison, principalement, de pertes hivernales.

Aujourd'hui, la population d'abeilles continue de décliner dans de nombreuses régions du monde, parmi lesquelles l'Europe et, plus particulièrement, la Belgique. Les apiculteurs ont constaté que 5 à 10 % de leurs abeilles mourraient chaque année. On observe en outre une diminution de l'aire de répartition des populations d'abeilles (Godfray et coll., 2015). Depuis 2006, les pertes annuelles se sont parfois élevées à 30 %, occasionnant des pertes économiques importantes pour les apiculteurs. Outre l'aspect économique, il convient de se demander dans quelle mesure la mort des abeilles doit être considérée comme l'indicateur d'un problème plus large de qualité et durabilité environnementales, susceptible de représenter une menace pour la santé humaine.

Il n'existe pas de réponse définitive à cette question. Différentes hypothèses circulent. Comme souvent dans le domaine des sciences, plus les hypothèses sur un sujet donné sont nombreuses, moins on dispose de connaissances basées sur des faits. Un nombre croissant de données identifient néanmoins les pesticides, et notamment les néonicotinoïdes et le fipronil, comme une cause du déclin des populations d'abeilles.

L'une des principales sources d'informations ayant conduit aux conclusions ci-dessus est l'« Évaluation Mondiale Intégrée de l'impact des pesticides systémiques sur la biodiversité et les écosystèmes » (WIA, 2014), qui est la première évaluation scientifique complète des données actuellement disponibles. Cette étude repose sur l'examen de 1121 études publiées dans des revues à comité de lecture et rapports publics. Ses résultats mettent en évidence les effets sur les écosystèmes et les espèces. Ils font état du déclin accéléré des populations d'invertébrés à l'échelle mondiale et de risques pour la biodiversité et les services écosystémiques. Ces résultats invitent à reconsidérer l'utilisation prophylactique à grande échelle des néonicotinoïdes et à appliquer un principe de précaution en renforçant les réglementations.

Le sujet abordé dans la WIA a aussi été traité dans une étude plus récente de *l'European Academies Science Advisory Council* intitulée « *Ecosystem services, agriculture and neonicotinoids* » (EASAC, 2015). Cette étude confirme pleinement les résultats de la WIA. Elle est utilisée dans le présent avis comme point de référence pour les données et les résultats de la WIA.

Par ailleurs, un examen de la littérature scientifique entrepris par Godfray et coll. (2014, 2015) vise à présenter les avancées récemment réalisées dans le domaine des sciences naturelles concernant les effets des néonicotinoïdes sur les insectes pollinisateurs.

Cette question est en cours de discussion au niveau européen. La *Task Force on Systemic Pesticides*, un groupe de travail sur les pesticides systémiques, se consacre actuellement à l'étude des néonicotinoïdes et de plusieurs autres insecticides ayant des effets similaires.
Parmi les substances examinées dans leur étude, sept sont approuvées dans l'UE comme substances dont l'incorporation dans des produits phytopharmaceutiques peut être autorisée par les États membres (c'est-à-dire qu'elles sont incluses dans l'annexe du Règlement d'exécution (UE) n° 540/2011 de la Commission (CE, 2011)). Ces substances sont le fipronil, l'imidaclopride, la clothianidine, le thiaméthoxame, l'acétamipride, le thiaclopride et le sulfoxaflor. En Belgique, l'incorporation dans des produits phytopharmaceutiques de toutes ces substances, à l'exception du sulfoxaflor, a été autorisée. Suite à une évaluation de leurs effets sur les abeilles à miel, l'approbation des quatre premières substances a été restreinte afin de protéger les abeilles (Règlement d'exécution (UE) n° 781/2013 de la Commission (CE, 2013b) et Règlement d'exécution (UE) n° 485/2013 de la Commission (CE, 2013a)).

Les substances soumises à des restrictions font actuellement l'objet de plusieurs autres évaluations au niveau de l'UE :

- Les Règlements (EU) n° 781/2013 (CE, 2013b) et 485/2013 (CE, 2013a) exigent des fabricants qu'ils transmettent de nouvelles informations dans un délai de 2 ans afin de confirmer l'innocuité des utilisations restreintes pour les insectes pollinisateurs.
- En 2015, l'Autorité européenne de sécurité des aliments (EFSA, acronyme *d'European Food Safety Authority*) a invité l'ensemble des parties intéressées à fournir de nouvelles données ; en novembre 2015, la Commission européenne a chargé l'EFSA d'évaluer les données communiquées.
- Les restrictions imposées par les Règlements (EU) n° 781/2013 et 485/2013 reposent sur une conclusion de l'EFSA suite à l'évaluation de toutes les utilisations des substances concernées pour le traitement des semences et comme granules ; l'EFSA a également évalué toutes les autres utilisations de ces substances, et en particulier les applications foliaires. Les conclusions de ces évaluations ont été publiées en août 2015 (EFSA, 2015a ; EFSA, 2015b ; EFSA, 2015c).
- L'approbation d'une substance active est normalement valable pendant une période de 10 ans et est renouvelable ; dans le cadre de ce renouvellement, une évaluation de la clothianidine et du thiaméthoxame a été entreprise en 2015 ; le fipronil et l'imidaclopride seront eux aussi soumis à une évaluation dans 1 ou 2 ans.
- Suite à une évaluation de toutes les informations concernant la neurotoxicité développementale de l'imidaclopride et de l'acétamipride, l'EFSA a adopté un avis scientifique à l'égard des valeurs de référence toxicologiques attribuées à ces substances.

Concernant le sulfoxaflor, une substance approuvée par la Commission européenne en 2015, des informations confirmatives relatives à l'évaluation des risques pour les abeilles doivent être fournies dans un délai de deux ans à compter de la date d'approbation. Ces données seront ensuite évaluées par l'État membre rapporteur.

Les résultats de ces évaluations sont en cours d'examen ou seront examinés par la Commission européenne et les États membres dans le cadre du comité permanent des végétaux, des animaux, des denrées alimentaires et des aliments pour animaux ; ces résultats pourront aboutir à des décisions réglementaires. Le présent avis recommande l'adoption par la Belgique d'une position scientifiquement fondée au sein du comité permanent et soutient par ailleurs les autorités fédérales belges compétentes concernant l'autorisation des produits phytopharmaceutiques.

Dans ce contexte, le Comité des directeurs du Service Public Fédéral Santé publique, Sécurité de la Chaîne alimentaire et Environnement a conseillé aux membres du gouvernement concernés, et en particulier au ministre de la Santé publique, au ministre de l'Agriculture et au secrétaire d'État à l'Environnement, de demander au CSS d'évaluer la WIA. Il a plus précisément été demandé au CSS de fournir un rapport consultatif contenant :

- une évaluation des études menées par le groupe de travail sur les pesticides systémiques publiées au cours de l'été dans la revue *Environmental Science and Pollution Research*, en accordant une attention particulière aux éléments suivants :
  - la rigueur scientifique de la méthodologie utilisée par les auteurs ;
  - les critères utilisés pour sélectionner les études examinées, et notamment ceux relatifs à leur pertinence et à leur fiabilité. Le document d'orientation de l'EFSA intitulé « *Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation* (EC) No 1107/2009 » (http://www.efsa.europa.eu/en/efsajournal/pub/2092.htm) peut être utilisé à cette fin ;
  - les doses auxquelles les organismes de test ont été exposés dans les études évaluées par le groupe de travail (et, plus précisément, si leurs niveaux sont comparables à ceux des doses auxquelles ces organismes sont susceptibles d'être exposés dans le cadre des applications conformes aux modalités définies pour la Belgique) ;
  - s'il s'avère que ces doses sont de niveau similaire : l'impact potentiel de l'utilisation des substances concernées sur la biodiversité en Belgique ;
  - les mesures de réduction des risques devant être incluses dans les autorisations pour réduire l'exposition des organismes non ciblés à un niveau acceptable ;
- l'impact possible sur la santé humaine d'une exposition suite à des utilisations conformes aux modalités d'application belges, en accordant une attention particulière à l'avis scientifique de l'EFSA sur le lien potentiel entre la neurotoxicité développementale et deux néonicotinoïdes – l'acétamipride et l'imidaclopride –, et au fondement scientifique de la proposition mentionnée dans cet avis de modifier les valeurs de référence toxicologiques.

Trois facteurs sont essentiels au moment d'évaluer la pertinence d'une étude (Maxim et van der Sluijs, 2013).

(-) La qualité scientifique, qui inclut des aspects techniques (les mesures sont-elles précises ?), méthodologiques (une méthode spécifique est-elle adaptée à l'utilisation prévue ?) et épistémologiques (les connaissances disponibles sont-elles suffisantes ?).
Les questions méthodologiques spécifiques et générales sont particulièrement importantes dans ce domaine. Avant de poursuivre, il convient de signaler que la WIA ne prétend ni être une « revue systématique » ni une « méta-analyse » ; elle se veut plutôt une analyse approfondie. Les questions relatives à la méthodologie utilisée par la WIA n'en demeurent pas moins pertinentes. Par ailleurs, les deux études complémentaires (étude de l'EASAC ; Godfray et coll., 2014, 2015) ne présentent pas les caractéristiques d'une méta-analyse.

(-) La qualité du processus de recherche à l'origine des connaissances accumulées et des avis d'experts utilisés pour évaluer son intérêt pour soutenir une action.
Ce facteur est lié aux compétences des chercheurs et des experts, à leurs expériences sur le terrain, à leur appartenance institutionnelle, à leur bien-être au travail, à leur contexte financier ainsi qu'aux relations entre les experts et avec d'autres parties prenantes.

(-) La qualité sociale, associée aux jugements de valeur qui influent sur la communication et l'utilisation des informations scientifiques par les experts et les parties prenantes dans les débats d'orientation.

Conformément à la mission du CSS, le présent avis tient uniquement compte du premier facteur. Il est structuré en trois parties :

- Une description de l'utilisation des néonicotinoïdes et du fipronil en Belgique.
- Un examen de la WIA, des conclusions qui en découlent et de la méthodologie adoptée à son égard. Une attention particulière sera accordée aux aspects relatifs à la biodiversité. Deux études complémentaires plus récentes (EASAC, 2014 ; Godfray 2014, 2015) sont également examinées, et notamment leur pertinence par rapport à la WIA.
- Une évaluation des effets des néonicotinoïdes sur la santé/des menaces que représentent les néonicotinoïdes pour la santé, portant plus particulièrement sur les aspects relatifs à la neurotoxicité.

Le présent avis propose dans sa conclusion des méthodes d'atténuation et de réduction des risques ainsi qu'une inscription dans un contexte réglementaire.

II. METHODOLOGY OF THE ADVICE

After analysing the request, the Board and the Chair of the working party on chemicals identified the necessary fields of expertise. An *ad hoc* working group was established including an interdisciplinary array of competences on pesticides, ecology and eco-toxicity, health and public health policy. The experts of this working group provided a general and an *ad hoc* declaration of interests and the Committee on Deontology assessed the potential risk of conflicts of interest.

This advisory report is based on published papers in the international scientific literature and European and Belgian reports until 1 December 2015. It discusses the essentials of a health risk assessment: hazard (both health and eco-toxicological), exposure and dose-response relationships. These elements allow concluding on the questions which were submitted to the Council. When it includes the opinion of the experts, this is specifically indicated.

Two authors of the WIA-study were heard.

The draft advice report was reviewed by a scientific expert. On the basis of her comments, the report was revised.

Once the advisory report was endorsed by the ad hoc working party and by the standing working group on chemicals, it was ultimately validated by the Board.

III. ELABORATION AND ARGUMENTATION

1. Neonicotinoids and fipronil

1.1 Chemical properties of the core substances and their metabolites

Table 1. List of active substances classified as neonicotinoids and/or having post synaptic activity (acetylcholine receptor agonists).
(PPP = plant protection product, vet subst = veterinarian substance)

Active substance	PPP*/biocide**
Fipronil	PPP-biocide (vet subst)
Imidacloprid	PPP-biocide (vet subst)
Thiamethoxam	PPP-biocide
Clothianidin	PPP
Acetamiprid	PPP-biocide
Thiacloprid	PPP

* Ref. Fytoweb (www.fytoweb.be),
**Ref. FPS (Federal Public Service) authorised biocides (http://www.health.belgium.be/eportal/Environment/Chemicalsubstances/Biocides_NEW/ListOfAuthorisedBiocides/index.htm#.VNoPyi73ZTs)

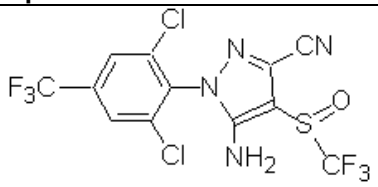
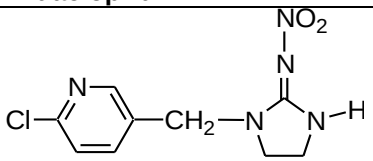
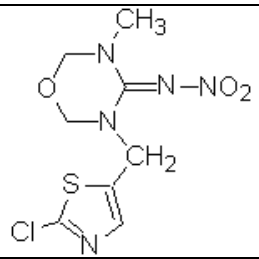
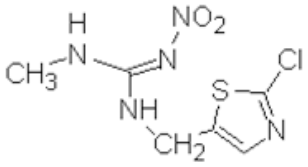
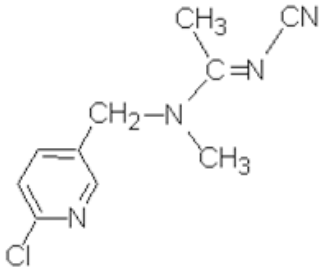
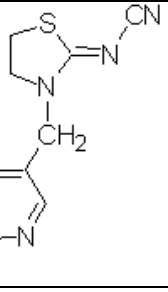
Fipronil	Imidacloprid	Thiamethoxam
		
Clothianidin	**Acetamiprid**	**Thiacloprid**
		

Figure 1. Structural formulas of the substances addressed by the advice.

Table 1 lists the 6 active substances which are addressed by this advice. General information and details on the chemical properties of these substances are presented in annex 1. Figure 1 provides the structural formulas showing the chemical relationships between these products. Four substances contain a chlorinated hexahedral ring. Imidacloprid, thiacloprid and acetamiprid share the toxicophore heterocyclic ring (the 6-chloro-3-pyridylmethyl moiety) while in thiamethoxam and its metabolite clothianidin, this moiety is replaced by a 2-chloro-5-thiazolyl group.

For classification purposes, the five chloronicotinyl compounds of interest are subdivided on the basis of the presence of a functional group at the other side of the molecule, either the nitroguanidines (imidacloprid, thiamethoxam and clothianidin) or cyanoamidines (thiacloprid, acetamiprid).Selected toxicity data related to consumption, worker exposure and eco-toxicity to aquatic life are provided in annex 2. Fipronil and neonicotinoids are mainly used as insecticides in crop protection. Fipronil and imidacloprid are also used as veterinary medication. Thiacloprid has next to insecticide properties, a molluscicide action. Except for fipronil most substances are reported to be systemically transported in crops. The mode of action is both: contact and stomach action. This means that the insect is intoxicated as a result of the intake (consumption) of the treated crop or through contact (uptake through the cuticle) with the treated crop surface. Fipronil and thiamethoxam are reported broad-spectrum insecticides. This means that these pesticides are toxic for a wide range of insects and hence may kill also non-target and beneficial insects like the honeybee.

In July 2015 sulfoxaflor was approved as pesticide by the EU. The WIA report (2014) elaborates on five neonicotinoids and fipronil. Therefore, unless mentioned otherwise, the environmental and health effects discussed in this advice only relate to the six substances (five neonicotinoids and fipronil) on the market before 2015.

Table 2. Ranking of substances according to their chemical properties (University of Hertfordshire, 2015)

	High	**Medium**	**Low**
Vapour pressure (P_v)	P_v > 1000 mPa	0.01 mPa < P_v < 100 mPa	P_v < 0.01 mPa
Water solubility (S)	S > 500 mg/L	50 mg/L < S < 500 mg/L	S < 50 mg/L
DT50	DT_{50} > 100d	30d < DT_{50} < 100d	DT_{50} < 30d
Koc	Koc > 4000	500 < Koc < 4000	Koc < 500
log K_{ow} (= log P)	log K_{ow}> 3	2.7 < log K_{ow} < 3	log K_{ow} < 2.7

Among the chemical properties of these substances (table 3), the most noticeable properties are:

- vapour pressure (Pv), indicating if and to which extent a substance is volatile, which causes inhalation exposure;
- water solubility (S), indicating if a substance is soluble in water and may contaminate the surface, ground- and drinking water;
- DT_{50}, indicating the persistence of a substance. DT_{50} stands for the degradation time or the time that has to pass to degrade 50 % of the initial dose. DT_{50} values vary according to the matrix where the substance is measured. DT_{50} values are commonly investigated for water (DT_{50} water) and soil (DT_{50} soil);
- Koc (Soil Organic Carbon-Water Partitioning Coefficient), indicating the absorption properties of a substance. Koc is the partition coefficient determining the amount of substance that adsorbs to the organic carbon part when a substance is dissolved in water;
- Kow (Octanol-Water Partitioning Coefficient) indicating the bio-accumulation properties. The octanol water partition coefficient is the distribution of the substance over the octanol (indicative for the bio matrix) and the water phase.

Table 3. Chemical properties of fipronil and neonicotinoids

		fipronil	imidacloprid	thiamethoxam	clothianidin	acetamiprid	thiacloprid
Log K_{ow}	pH7, 20°C	3.75	0.57	-0.13	0.905	0.8	1.26
Vapour pressure	mPa	0.00037	4E-07	6.6E-06	2.8E-08	0.001	3E-07
Solubility	mg/L	1.9	610	4100	340	4250	185
DT_{50} soil	days	65	174	39	121	3	18
DT_{50} water/sed*	days	68	129	50	56.4	5.8	19
K_{oc}*	mL/g	577	225	56.2	160	106.5	615

*Database lab Crop Protection Chemistry, UGent

Based on the reported vapour pressures all substances are classified as (very) low volatile compounds. The vapour pressure is for most substances far below the reference value of 0.01 mPa. This means that exposure to these compounds through the air is minimal. Only (professional) exposure to inhaled dust carrying the active pesticides is considered significant.

For all compounds, except for fipronil, solubility in water is medium to high. This is also reflected by the systemic character of these compounds inside plants, in which water is transported from the roots to the leaves, where the water is vaporized. It is likely that the compounds are transported with the water phase in the plants. The water solubility ranks from 1.9 mg/L for fipronil up to 4.25 g/L for acetamiprid. The latter value exceeds almost 10 times the reference value of 500 mg/L, which results in the classification 'high' in table 2.

Investigating the bio-accumulation properties of these compounds, it seems that except for fipronil, the log Kow is below 1.3. This means that all neonicotinoids according to this classification system have low potential to bio-accumulate in the food chain. Fipronil however is classified as strongly bio-accumulative.

1.2 Environmental characteristics (persistence) and fate

The major parameter to evaluate the environmental fate, is the soil half-life time of the substance (DT_{50}). Soil is the major receiving compartment when these products are applied during the cropping season. Low persistent substances are broken down within 30 days. High persistent products are products with a half-life time over 100 days. 100 days reflects a growing season of 3 months during which the substance degrades, otherwise problems may occur during the next growing season. As shown in table 3, neonicotinoids as imidacloprid and chlothianidin are highly persistent in soil and/or in water. However, degradation products may be relevant as well and may have a longer half life time than the parent active substance. Thiamethoxam for instance has a medium persistence in soil, but clothianidin, which is highly persistent in soil, is a major degradation product of thiamethoxam. The degradation of acetamiprid, with its very short half life time, is also leading to the presence of highly persistent and probable toxicologically relevant metabolites.

The sorption capacity of these substances to the organic matrix of the soil is intermediate to low. Only for fipronil and thiacloprid the Koc (slightly) exceeds the lower limit for the medium reference value. This means that, except for fipronil and thiacloprid, most substances, taking into account their persistence, are at risk of being transported through the soil matrix to a non-target zone, for instance the groundwater. Based on their chemical Koc value, the compounds thiamethoxam, clothianidin and acetamiprid behave rather mobile in the environment. Data on the exposure routes of neonicotinoids and fipronil are summarized in box 1.

Box. 1. Exposure routes

Exposure Route	
Air	Based on the vapour pressures, all neonicotinoids are classified as (very) low volatile compounds. Occurrence of neonicotinoids in air as a vapour is not expected. Exposure during leaf application by workers, bystanders and residents may occur. This is because of droplet drift of the spray. Exposure during seeding by workers, bystanders and residents may occur. This is because of dust drift of fine particles released during seeding.
Water	Except for fipronil, solubility of neonicotinoids in water is medium to high. Occurrence of neonicotinoids in water is expected.
Soil	Some of the neonicotinoids are highly persistent. They remain a long time in soil. Exposure through soil contact is expected.
Food	Neonicotinoids are applied on fruit and vegetables. They can be detected in food.

1.3 Use

1.3.1 Use as plant protection products and as biocides or veterinary product

Table 1 shows that neonicotinoids are not exclusively crop protection chemicals (PPP). The exposure to these substances in Belgium should also include their biocidal and veterinarian use.

Some neonicotinoids (PPP) are not only used in agriculture by professionals, they are also used in private gardens, in parks and on sport terrains. The professional (authorisation number "*****P/B") and the private use (authorisation number "*****G/B") products available on the Belgian market are listed in annex 3.

Neonicotinoids can be applied as leaf treatments, as seed treatments and as soil treatments. Treated seed acts as a delivery mechanism for some of the neonicotinoids: seed treatments allow acting on early season insects and diseases at the time of planting and thereafter, while minimizing exposure of humans, animals and environment. The plant protection product is applied once in the season and the low dose needed, compared to leaf treatment, is appealing when trying to avoid excessive use of chemicals on crops on an almost weekly basis. Disadvantages of seed treatment include the release of the fine dust particles during seeding, and the persistence and systemic transport of the neonicotinoids to the leaves (guttation) and blossoms of the plants later on during the season.

1.3.2 Widespread use; gross production/consumption figures; trends

The yearly updated environmental report of Flanders (MIRA, 2014), provides sales and use figures of pesticides in Flanders over time. It relates these sales and use data to the impact of pesticides in Flanders on aquatic life. In this work, the sales figures of the neonicotinoids show a rather increasing trend until 2013 (annex 4). Since 2013 the use of major neonicotinoids has been restricted. This restriction is reflected in the downward trend of the sales figures from 2013 on.

Sales figures differ from use figures. A product can be sold in a particular year, but is not necessarily used during the same period. The use figures for Flanders are based on the farm accountancy data network of about 700 farmers. As mentioned in section 1.3.1 neonicotinoids are also used for non-agricultural purposes.

Similar use figures are available for Wallonia. The Walloon data do not take seed treatment into account. Although the data are incomplete, combining the Flemish with the Walloon data (2010, 2011 and 2012) provides insight in the origin of the difference between sales and use figures.

During the period 2010-2012, almost 10 times more imidacloprid and thiamethoxam was sold in Belgium than the amount used by the farmers. Clothianidin was sold in Belgium (ca. 7000 kg) but was not used. Fipronil on the opposite was not sold in Belgium but was still used (ca. 1000 kg). An interesting co-incidence was found for acetamiprid and thiacloprid, of which the sales and the use figures did not show major discrepancies. Although it is likely that still a remarkable amount of neonicotinoids was used for seed treatment in Belgian farms at that time, the discrepancy between the sales and use figures is too high. This confirms the statement of the crop protection chemistry industry that in Belgium part of neonicotinoids is sold for seed treatment, while the treated seeds are exported.

1.3.3 Regional and sectorial distribution

Tools exist predicting the distribution of pesticides in the Belgian environment. The WEISS model developed by the *Vlaamse Instelling voor Technologisch Onderzoek* (VITO) allows estimating the surface water contamination from contaminants including pesticides. The WEISS-model has been used by the BEE-Happy project funded by the Flemish Fund IWT (*Agentschap voor Innovatie door Wetenschap en Technologie* / Agency for Innovation by Science and Technology) (2013-2014). One of the outcomes of the project (in preparation) are maps showing the distribution of the use of the neonicotinoids across Flanders. Imidacloprid shows a quite homogeneous distribution across the region, for thiamethoxam the use is localized in the area where particular target crops are grown.

The yearly environmental report (MIRA, 2014) of the Flemish Government calculates the impact of the use of pesticides. A distinction is made between the use by agriculture, horticulture and non-agricultural use (see some of the figures related to the neonicotinoids in annex 4). Within agriculture, the pesticide use over the major crops is calculated to evaluate the environmental impact on a crop by crop basis. This provides an indication of the use of a particular pesticide in a defined region. A comparable outcome with the WEISS maps is expected.

1.3.4 Distribution over environmental compartments; effects on pollination, ecosystem resilience, community diversity

Neonicotinoids were analysed in Flanders in surface waters (*Vlaamse Milieumaatschappij*, 2015 – table 4). In 90 % of the sampling points imidacloprid was found, in 44 % of the sampling points thiamethoxam and in 26 % clothianidin. Imidacloprid is mostly found during August and October but also in May. It is detected in small and major water bodies. The highest concentration observed is 600 ng/L. Thiamethoxam is not frequently found but shows locally high values. The highest observed concentration exceeded 1400 ng/L. The concentration of chlothianidin in water was below 65 ng/L. Compared to the MAC (maximum allowable concentration) calculated based on toxdata from fish, daphnia and algae, these concentrations do not offer reasons for concern. All concentrations are below the eco-tox reference values. The ecotoxicological data used for comparison are however subject to discussion. The environmental agency of Flanders (*Vlaamse Milieumaatschappij*, 2015) selected different values, resulting in much lower threshold values. These guidelines involve the complete biodiversity of the aquatic system. They take also toxicity of these insecticides on aquatic insects into account, which explains the guidelines for imidacloprid are exceeded (sometimes by 100 %) at all positive sampling points.

Table 4 Neonicotinoïdes in surface water in Flanders

Parameter	number of measuring stations with >1 detection	number of sampled measuring stations	percentage
Clothianidin	24	92	26 %
Imidacloprid	83	92	90 %
Thiamethoxam	40	92	44 %

1.3.5 Consumption figures

Annex 5 shows the human exposure to neonicotinoids as described by the report of the Federal Agency for the Safety of the Food Chain (FASFC) (in press). This study based on monitoring data, shows that the chronic or long term exposure of the Belgian consumer as a result of the consumption of fresh products is safe for the years considered. 34 pesticide residues were selected based on their detection frequency and representativeness. 3 neonicotinoids, namely acetamiprid, imidacloprid and thiacloprid, are among this set of 34 pesticides.

A deterministic risk assessment concludes that the exposure of an average adult consumer to most residues (neonicotinoids included) is at least 100 times below the acceptable daily intake (ADI). No specific risk is calculated for consumers eating a lot of fruits and vegetables (97.5 percentile exposure or P97.5). Indeed, the graphs in annex 5 show for thiacloprid a 97.5P exposure for all cases less than 5 % of the ADI; for acetamiprid and imidacloprid the 97.5P exposure is always lower than 1 % of the ADI.

The study also concludes that there is no direct correlation between the sold volume of a pesticide, the frequency of its detection or the exposure to the pesticide residues by food consumption.

1.4 Assessment/conclusions

Neonicotinoids are used in agriculture (professional and non-professional use). They are also used as biocide and as veterinary drugs. None of the neonicotinoids is volatile: therefore exposure through inhalation is unlikely to occur (apart from pesticides bound on dust). Some compounds or their metabolites are persistent: they will stay in the environment for a long time after their application. As solubility in water is high and sorption to the soil matrix for a few components is low, transport through the soil matrix form the treatment zone to the non-target water zone should be expected.

In contrast to leaf treatment, seed treatment minimizes human and environmental exposure.

The amount of neonicotinoids applied in Belgium can be partially estimated based on sales figures or on use figures. There is uncertainty on estimating the use of neonicotinoids as seed treatment. The figures show an increase in the sales until 2013. After the 2013 restriction (for use as seed treatment and selected foliar and soil treatments) the sales figures decreased. The use figures based on accountancy data reflect more accurately the really applied quantities, compared to the Belgian sales figures which do not list import/export data of treated seeds.

In Flemish surface water neonicotinoids are detected at levels close to the detection limits of the analytical equipment (10 ng/l). No similar data are available for Wallonia. No European norm for the neonicotinoids in surface water exists, allowing an environmental risk assessment.

Finally, according to the advisory report of the FASFC on human exposure to residues of plant protection products (PPP) in fruits and vegetables (FASFC, 2015) the measured values of neonicotinoids in fruits and vegetables are below the acceptable daily intake (ADI). Similar data on human exposure to biocides and to private use of PPP (gardening) in Belgium currently lack.

2. Mechanisms and environmental hazards

2.1 General

2.1.1 Mode of action

Most insecticides are nerve poisons acting on the voltage-dependent sodium channel (e.g. pyrethroids), the γ-aminobutyric acid (GABA) receptor (e.g. organochlorines, fipronil), the cholinergic system as inhibitors of acetylcholinesterase (e.g. organophosphates or carbamates) or agonists at the nicotinic acetylcholine receptor (nAChR) (Casida and Quistad, 1998). Imidacloprid is a powerful agonist of the nicotinic acetylcholine receptor, which binds specifically on the alpha subunits (Matsuda et al., 2001). Nicotinic acetylcholine receptors are ligand gated ion channels involved in the synaptic transmission of the central nervous system.

A classical nAChR agonist, as nicotine, was used for centuries to control sap-sucking insects despite its relatively low effectiveness and its high toxicity to mammals.

In contrast, the neonicotinoids, which are also nAChR agonists, are more toxic to insects and relatively less toxic to mammals, providing an example of selective toxicity (Yamamoto and Casida, 1999). Most neonicotinoids undergo metabolic alterations at multiple sites. It has been demonstrated that during early development stages some metabolites may show a higher activity on mammalian than on insect receptors (Chao and Casida, 1997) and that the toxicity of the analogues and metabolites of neonicotinoids in mammals may involve action at multiple receptor subtypes with selectivity conferred by minor structural changes (Tomizawa and Casida, 1999). However, since biotransformations in mammals might involve activation reactions, but largely detoxification mechanisms (Tomizawa and Casida, 2005) *in-vivo*, it is generally accepted that neonicotinoids are less toxic to mammals than to insects. It is unclear if the toxicity of imidacloprid in mammals is due to the parent compound or the de-nitro metabolite (which enters the brain following direct intraperitoneal administration in mice).

The neonicotinoids not only show a high affinity for the receptor; important physicochemical properties include non-ionisability and medium to high water solubility (Table 2 and 3).

The nicotinic acetylcholine receptors (nAChR) are neurotransmitter-regulated ion channel complexes, which are responsible for rapid synaptic transmission. They are neuron receptor proteins that signal muscular contraction following a chemical stimulus and form ligand-gated ion channels in the plasma membranes of selected neurons. In insects, the cholinergic system is limited to the central nervous system, and the nAChR acts as the most important target for neonicotinoid action.

Structurally, 5 single subunits (typically 2α and 3β) structure a pentameric transmembrane protein with a central cation-permeable ion channel. Amino acid sequence comparisons showed that insect receptors have a similar structure as vertebrate receptors (Nauen et al., 2001).

2.1.2 Toxicology

The neonicotinoids imidacloprid, thiacloprid and acetamiprid share the toxicophore heterocyclic ring (the 6-chloro-3-pyridylmethyl moiety) while in thiamethoxam and its metabolite clothianidin, this moiety is replaced by a 2-chloro-5-thiazolyl group. For classification purposes, the five chloronicotinyl compounds of interest are subdivided on the basis of the presence of a functional group at the other side of the molecule, either the nitroguanidines (imidacloprid, thiamethoxam and clothianidin) or cyanoamidines (thiacloprid, acetamiprid).

These neonicotinoids were selected because both nitroguanidine and cyanoamidine neonicotinoids are most active against insects. For imidacloprid analogues for example, nitroimine (=N–NO_2) toxicophores conferred more insecticidal activity than their cyanoimine (=N–CN) counterparts (Nauen et al., 2001).

2.2 Effects

2.2.1 WIA study, EASAC review, and the Godfray et al. studies

This section summarizes the environmental effects of neonicotinoids and fipronil as reported by three complementary studies:

- the WIA study (2014) which provided the immediate trigger for this advice, both its methodology and its main results are reviewed;
- a literature review by the EASAC (2015) of the relation between agriculture and ecosystem services;
- a literature review initiative by mainly British scientists aiming at providing a restatement of the natural science evidence base concerning neonicotinoid insecticides and insect pollinators (Godfray et al, 2014; Godfray et al., 2015).

The section only summarizes the results and outcomes of these studies (cf. box 2, 3 and 4). Details and examples are provided in the references and their annexes. The discussion of these data, including their importance for ecosystems and human exposure in Belgium, is provided later on in this advice.

Box. 2. WIA methodology and results

I. Methodology used by WIA
As a whole the WIA-study is based on the review of 1121 papers and reports dealing with neonicotinoids (with in specific parts a special focus on selected substances as imidacloprid and clothianidin) and fipronil. The study classifies its reviews according to 8 main areas affecting animal groups and ecosystems. Concerns are raised on the selection of the documents on which WIA is based. Of the 8 reports constituting WIA, only one (Gibbons et al, 2014) handles reference selection criteria allowing to classify the study as a systematic review[3]. The other 7 studies are vague and lack a sufficient methodology description, not allowing considering them as systematic and/or meta-reviews. This lack of information on the selection of the reviewed studies, in combination with insufficient data on how the included papers and reports are screened, impairs the scientific validity of the WIA-review. Of notice however is that WIA does not claim the "meta-analysis" or "systematic review" label. Rather it is a "comprehensive scientific assessment" or "comprehensive analysis", a qualification for which the scientific criteria are less clear. During its review of the WIA assessment the SHC did not find any indication of bias or over-interpretation. Moreover the council points to the fact that the WIA-results provide similar evidence as the conclusions of the (methodologically stronger) European Academics Science Advisory Council (EASAC, 2015) report.
II. WIA results
II.1. Systemic pesticides: trends, uses, mode of action and metabolites (Simon-Delso et al., 2014)
– Neonicotinoid pesticides are the most widely used class of insecticides worldwide. They are used in agriculture, horticulture, orchards, forestry, veterinary applications and fish farming. They currently account for approximately one third of the world insecticide market.

[3] The terminology used to describe systematic reviews and meta-analyses has evolved over times and varies between fields.

A ***systematic review*** is a review of a clearly formulated question that uses systematic and explicit methods to identify, select and critically appraise relevant research, and to collect and analyze data from the studies that are included in the review (Cochrane handbook for reviews, glossary).

The key characteristics of a systematic review are: (a) a clearly stated set of objectives with pre-defined eligibility criteria for studies; (b) an explicit, reproducible methodology; (c) a systematic search that attempts to identify all studies that would meet the eligibility criteria; (d) an assessment of the validity of the findings of the included studies, for example through the assessment of risk of bias; and (e) a systematic presentation and synthesis of the characteristics and findings of the included studies (Liberati et al., 2009).

A systematic review may or may not be completed by statistical methods to quantitatively synthetize results.

Meta-analysis refers to these statistical methods to summarize the results of independent studies. However, meta-analysis is not always possible or desirable, due to clinical, methodological or statistical differences across the included studies.

Systematic review and meta-analysis are multiple steps procedures including study identification, study selection, data extraction, data analysis including evaluation of heterogeneity, statistical pooling, assessment of publication bias, sensitivity analyses and finally data interpretation.

Advantages of systematic reviews and meta-analysis: systematic review uses explicit, systematic methods that are selected with a view to minimizing bias, thus providing more reliable findings from which conclusions can be drawn and decisions made. By combining information from all relevant studies, meta-analyses can provide more precise estimates of the effects of health care than those derived from the individual studies included within a review. They also facilitate investigations of the consistency of evidence across studies and the exploration of differences across studies.

- Fipronil (a phenyl-pyrazole compound) and neonicotinoids show similarities in their toxicity, physicochemical profiles and presence in the environment.
- At their introduction during the 1970s there was no known resistance to the products under study; their physicochemical properties include advantages over previous generations of insecticides and they have assumed reduced operator and consumer risks. These are reasons for their application success.
- They are taken up by the roots or leaves and translocated to all parts of the plant ("systemic character") which results in exposure of herbivorous insects.
- They are mainly found in soil and water.
- Their toxicity persists for various periods of time. The most significant effects result from their persistence. E.g. imidacloprid has a half-life time in the soil of ca. 6 months.
- Neonicotinoids mimic the action of neurotransmitters, while fipronil inhibits neuronal receptors. By stimulating neurons, they lead to the death of target invertebrates. Neonicotinoids share greater affinity towards arthropod acetylcholine (ACh) receptors than towards those of mammals and other vertebrates.
- They have lethal and sub-lethal effects on non-target organisms, including insect predators and vertebrates.
- Synergistic effects with other pesticides have been documented.
- Metabolites can be toxic by their own.
- Taken together these elements (and in particular the last 5 in the list) neonicotinoids and fipronil offer significant risks to the environment. The current literature shows that persistent, low concentrations of these pesticides pose serious risks of undesirable environmental impacts.

II.2. Environmental fate and exposure to neonicotinoids and fipronil (Bonmatin et al., 2014)

- Neonicotinoids and fipronil are among the most widely used pesticides in the world. They are used as foliar spray, seed treatments and seed drenches.
- Environmental contamination occurs via a number of routes including dust from treated seeds, soil, and surface water. Overall, there is strong evidence that soils, waterways and plants in agricultural environments are contaminated with varying concentrations of neonicotinoids and fipronil. International reports have been published showing that these concentrations exceed eco-toxicological limits.
- Neonicotinoids are highly toxic to invertebrates because of their systemic nature. They are soluble in water and have a variable although often long persistence time in the environment (e.g. the half-lives of neonicotinoids in soil can exceed 1000 days, which results in cumulative effects as a result of repeated use). (These findings complement the crop protection chemistry data summarized in table 3.)
- Breakdown of the products under study results in toxic metabolites, though concentrations of these are rarely measured in the environment.
- The widely spread presence of these products provides multiple routes of exposure for non-target animals. Studies of honey bee colonies point to a lifelong routine use and chronic exposure to neonicotinoids and fipronil, and their metabolites (in general in the experimental 1-100 ppb range). In spring the use of seat-coating insecticides for crops results in a risk of acute intoxication of bees and other pollinators. Often the pesticides under study are mixed with other pesticides, some of which are known to act synergistically with neonicotinoids. For most other non-target animals data on effects lack.
- This environmental contamination will have impacts on the functioning of ecosystems and their services. The development of alternatives to the use of neonicotinoids and fipronil seems imperative.

II.3. Effects on non-target invertebrates (Pisa et al.; 2014)

- The effects of neonicotinoids and fipronil on terrestrial, fresh water and marine invertebrates are summarized from almost 400 published papers and reports.
- Special attention is given to honeybees (*Apis mellifera*) as a pollinator. Also effects on butterflies, moths, earthworms, bumblebees, solitary bees and other invertebrates were

considered. Most information is provided by *in vitro* experiments. There is a need for new and improved methods to define adverse effects on a variety of fauna groups.
- Few information is available on freshwater and marine species.
- Effects on terrestrial species range from organismal toxicology and behavior effects, to population effects. Neonicotinoids exhibit a very high toxicity to a wide range of invertebrates, particularly insects, resulting in both lethal and sublethal impacts.
- On most invertebrate species the effects have not been studied, resulting in major uncertainties. This comment applies also to other pesticides and even to man-made chemicals in general.
- Current concentrations in the environment frequently exceed the lowest observed adverse effect concentrations. Therefore large-scale and wide ranging negative biological and ecosystem impacts are to be expected. It is suggested to tighten regulations on the use of neonicotinoids and fipronil.

II.4. Effects on vertebrate wildlife (Gibbons et al., 2014)

- The study focuses on direct (e.g. toxic) and indirect (e.g. food chain) effects of imidacloprid, clothianidin and fipronil in mammals, birds, fish, amphibians and reptiles. The results are based on a review of 150 studies.
- Imidacloprid and fipronil were found causing lethal effects in many birds and most fish.
- At sub-lethal doses, all three substances exert genotoxic and cytotoxic effects; they impair the immune system, cause reduced growth and affect reproduction. These effects occur at concentrations in orders of magnitude below those causing lethality.
- Also seed treatment of crops poses health risks to granivorous small birds, in particular to sensitive species.
- The concentration of fipronil in surface water may be sufficiently high to harm fish.
- There is a paucity of data on indirect effects. Case studies point to impaired growth in fish and population decline in lizards.
- On mechanisms the study refers to the systemic nature of the studied substances.
- The study points to the need of considering in an integrated way the direct and indirect effects on vertebrate wildlife (see also box 5 on effects of neonicotinoids on birds).

II.5. Ecosystem functioning and services risks (Chagnon et al., 2014; Daily and Korps, 2015)

- Ecosystem services are about valuing the service potentials, benefits and use values that well-functioning ecosystems provide to humans and the biosphere.
- Neonicotinoids and fipronil are found in all environmental compartments, but mainly in soil and water. These environmental media provide essential resources to support biodiversity, but are threatened by the presence of the substances under study.
- Specific ecosystem services impacts have focused on the negative impacts on pollination of food crops. The foods animals pollinate are fruits, vegetables, nuts, and seeds of which dietary deficiency confers risk of non-communicable diseases, including cardio-vascular disease, diabetes and lung cancer. Altogether, 35% of the global food volume derives from animal pollinated crops.
- Ecosystem services provided by target and non-target organisms are wider than pollination. They also include the regulation of soil and water quality, pest control, ecosystem resilience and community diversity. In particular microbes, invertebrates and fish are essential in maintaining healthy ecosystems.
- Systemic pesticides have negative impacts on decomposition, nutrient cycling, soil respiration and invertebrate populations, all sustaining healthy communities and integer ecosystems.
- Threatening pollination and other ecosystem services has economic impacts and raises cultural concerns which are currently difficult to quantify.
- Also these data advocate improved sustainable agricultural practices, including a restricted use of systemic pesticides.

II.6. Conclusions of the WIA study (van der Sluys et al., 2014)

- The increasing global reliance on the use of persistent and potent neurotoxic systemic insecticides as neonicotinoids and fipronil raises concerns on their impact on biodiversity, ecosystem functioning and services.
- Their present use combined with their properties has resulted in widespread contamination of agro-ecosystems, soils, freshwater, wetlands and non-target vegetation in estuarine and coastal ecosystems.
- Examples have been described showing how the use of neonicotinoids results in unnecessary contamination of the environment, thereby increasing risks to non-target organisms and to pesticide resistance development.
- More evolved approaches to good agricultural (and related) practices as integrated pest management should consider all relevant and available information to make informed management decisions.
- As these data are recent and have been insufficiently taken into account during the market authorization of these products. The regulatory framework failed to assess the individual and joint ecological risks resulting from their use in combination with other pesticides and environmental stressors.
- Ecological risk assessment thus far did not consider the various documented inter-actions (additivity, synergism) with other environmental stressors.
- The current authorisation process impairs re-assessment, delivers no limits on total amounts of pesticides applied and does not include mechanisms reducing the total use of the authorized products.

The controversy over the effects of neonicotinoids on honey bees prompted also the EASAC (2015) to a literature review of the relation between agriculture and ecosystem services and what is known about their economic value in the EU Member States.
The report shows a number of parallels with the worldwide WIA study including its general logic, its focus on honey bees and other pollinators, its scope on agriculture, both target and non-target organisms, and ecosystem services. In contrast to the WIA study, EASAC explicitly analyses the relevance of the merely recent (since 2011) scientific data for the EU policy.

Box 3. EASAC-review (EASAC, 2015)

The EASAC experts concluded:
1. There is an increasing body of evidence that the widespread prophylactic use of neonicotinoids has severe negative effects on non-target organisms that provide ecosystem services including pollination and natural pest control.
2. There is clear scientific evidence for sub-lethal effects of very low levels of neonicotinoids over extended periods on non-target beneficial organisms. These should be addressed in EU approval procedures.
3. Current practice of prophylactic usage of neonicotinoids is inconsistent with the basic principles of integrated pest management as expressed in the EU's Sustainable Pesticides Directive.
4. Widespread use of neonicotinoids (as well as other pesticides) constrains the potential for restoring biodiversity in farmland under the EU's Agri-environment Regulation.

The Godfray et al. studies are based on a literature review of recently published papers. Conclusions are summarized in box 4. Papers were assessed on four aspects:
- experimental studies and field data;
- expert opinions;
- supporting evidence;
- projections.

Box 4. Review by Godfray et al. (2014, 2015)

The participating experts concluded:
- On *exposure of pollinators*: There are several proven pathways through which pollinators may be exposed to neonicotinoid insecticides applied as seed treatments (or in other ways). Some quantitative information on these exposure routes is available. Most exposure will be at sub-lethal levels from foraging on seed treated plants, the most important exception being contamination from dust at the time of planting. Better quantitative data on typical concentrations (in different environmental components) is desirable. - On *laboratory studies and sub-lethal effects*: Sub-lethal neonicotinoid exposure can affect many aspects of pollinator behavior and physiology. Sub-lethal effects at field-realistic doses are now established, but their consequences for pollinator populations and pollution are still unclear. - On *neonicotinoid residues in pollen, nectar and wax on the field*: (Low levels of) neonicotinoids can be detected in wild pollinators as well as in honeybee and bumblebee colonies. Data are few and restricted to a limited number of species. - On *field experiments*: Evidence accumulates that sub-lethal exposure to neonicotinoid insecticides, chiefly but not exclusively at the higher end of what is likely to be experienced in the environment, can affect foraging and other behaviors in the field. - On *(policy) consequences*: There still remain major gaps in our understanding of how pollinator colony-level and population processes may dampen or amplify the lethal or sub-lethal effects of neonicotinoid exposure and their effects on pollinator services. There is still a limited evidence base to guide policy makers on how pollinator populations will be affected by neonicotinoid use.

2.2.2 Non-target species – Vertebrates

There are increasing indications that imidacloprid is more toxic than previously thought and that it also has a stronger effect on mammalian nAChRs. Genotoxic effects have been shown in rats (Karabay & Oguz, 2005; Demsia et al., 2007).

Studies involving various animal species such as White leghorn cocks (Siddiqui et al., 2007), mice (Badgujar et al., 2013) and calves (Kaur, 2006) have reported liver damage and immunotoxicity (Badgujar et al., 2013). Reports on eggshell thinning, reduced egg-laying and altered incubation periods suggest that imidacloprid disrupts the endocrine balance (Matsuda et al., 2001; Berny et al., 2006).

At 10 µM, imidacloprid acts as an agonist of nAChRs in rat pheochromocytoma (PC12) cells (Nagata et al., 1998). It alters the membrane properties of stellate cells in the nucleus cochlearis ventralis in mice exposed for even less than one minute to a concentration of 10 µM (Bal et al., 2010). In humans, imidacloprid is believed to bind to the α4β4 nAChR subtype in particular (Li et al., 2011; Tomizawa & Casida, 2000). Another study reported that, in rats, imidacloprid has the same excitatory effects as nicotine on nAChRs in the cerebellar neurons at concentrations over 1 µM (Kimura-Kuroda et al., 2012, see evaluation below). In addition, imidacloprid builds up in the brains of mice following intraperitoneal administration (Lee Chao & Casida, 1997). Administering a single dose of 337 mg/kg/day (74 % of the median lethal dose (LD50)) on day nine of gestation results in sensorimotor deficits, an increase in cerebral acetylcholinesterase (AChE) activity and an increase in glial fibrillary acidic protein (GFAP) immunostaining in the motor cortex and in the dentate gyrus on day 30 after birth (Abou-Donia et al., 2008). Studies in rats show conflicting reports concerning the No Observed Adverse Effect Level (NOAEL) for the sub-acute to sub-chronic administration of imidacloprid. An initial study observed that the oral administration of imidacloprid at the lowest concentration (45 mg/kg/day) for 28 days resulted in a lower spontaneous locomotor activity (SLA), pain threshold, AChE, creatinine kinase (CK), alkaline phosphatase (AKP), lactate dehydrogenase (LDH) and antioxidant enzymes, whilst the lipid peroxidation (LPO) was on the rise (Lonare et al., 2014). Also pathological alterations in the brain were described. A series of experiments which involved administering imidacloprid orally to female rats for 90 days found that a dose of 20 mg/kg/day resulted in a lower weight gain, hepato- and nephrotoxicity, increased oxidative stress, hormonal changes, and anatomopathological alterations in the ovaries, as well as a reduced AChE-activity and a lower SLA with pathological alterations in the brain. These effects were not found in these studies at a dose of 10 mg/kg/day (Kapoor et al., 2011; Kapoor et al., 2010; Bhardwaj et al., 2010). In another study, in which imidacloprid was administered orally for 60 days, there were strong indications of liver toxicity at 20 mg/kg/day, but also indications of liver toxicity at 10 mg/kg/day (Vohra et al., 2014). Yet another study, in which Wistar rats were exposed between day 6 of gestation and day 42 after birth, found that age-related, dose-dependent developmental immunotoxic effects of imidacloprid occurred down to the lowest dose tested, *viz.* 10 mg/ kg/day (oral intake first by the mother and then by the animal itself) (Gawade et al., 2013). A subsequent study discovered that the reproductive system in rats was affected following the oral administration of a dose of just 0.5 mg/kg/day for 90 days (Bal et al., 2012a; Bal et al., 2012b). In a final study, imidacloprid induced immunotoxic effects after an oral intake of just 0.21 mg/ kg/day for 28 days (Mohany et al., 2012).

In conclusion there are increasing indications that imidacloprid can cause neurological damage to mammals at concentrations that are much lower than previously thought. However, there is a great deal of uncertainty as regards the NOAEL for sub-chronic to chronic administrations.

Box. 5. Effects of neonicotinoids on birds

Not only bees; also vertebrates are target species.

The widespread occurence of pesticides in our ecosystems raises concerns about the exposure of the systems and the organisms. It also impairs finding control areas for studying the effect of neonicotinoids.

Just like the WIA report, the EASAC report is a hazard assessment, with elements for evidence concerning sublethal effects and effects on biodiversity. Studies on the effects of neonicotinoid use in seed coatings on farmland birds on the one hand and the effects of their presence in surface water on insect populations and insectivorous birds on the other hand, provide illustrations.

Since several decades industry and authorities provide farmers with recommended application rate guidelines. Regulations are made in a way that those chemicals are supposed to be harmless for human and animal health and not to generate unacceptable risks for the environment. But one might ask the critical question if those recommendations are that safe for the biosphere.

A study (Lopez et al., 2015) in Environmental Research reports on the effects of exposure of farmland birds to the neonicotinoid imidacloprid on mortality, breeding investment and offspring immunity. Indeed, farmland birds may be exposed to toxic amounts of insecticides by ingestion of treated seeds. In an experimental setting, the researchers exposed adult partridges to two doses of imidacloprid: the recommended application rate for cereal seed coating and 20% of this rate, through imidacloprid treated wheat seeds. The mortality in the recommended application rate group of adult partridges was 100%, occurring faster in female birds than in males. This gender effect needs further research given its importance for species demography. The analyses of the liver revealed an accumulation of imidacloprid during exposure time. This may be very useful for field studies and risk assessment as levels of imidacloprid in the liver could correlate with their exposure. Although the group exposed to 20% of the recommended application rate showed no significant change in mortality rates, sub-lethal effects were seen among others on the level of immunosuppression (depressed T-cell immune response in chicks). This study shows that one has to realize and be aware of the fact that application rates stipulated by regulations are not always safe and can harm animal health.

The use of neonicotinoids in general, and imidacloprid in particular, is not only detrimental for insects, but also for birds. Indeed, invertebrates constitute an important part of the diet of many bird species during the breeding season and are indispensable for raising offspring. Hallmann et al. (2014) was the first establishing a strong correlation between the presence of imidacloprid in the environment and the decline in sparrow populations in the Netherlands. During the period 2003-2010, populations reduced on average by 3.5 % each year from an imidacloprid concentration of surface-water exceeding 20 nanograms per litre, even after correcting for spatial differences in land-use changes. Additional analyses revealed that this spatial pattern of decline appeared only after the introduction of imidacloprid in the Netherlands from 1990 on. The study of Hallmann et al. suggests that the observed declines in insectivorous birds could be associated with high neonicotinoid concentrations in the surface water.

Moreover, the pesticides issue is economically relevant in many ways: the pesticides industry is a billion business on the one hand, but on the other hand a steep decline in honey bee and other pollinators populations, together with a loss in biodiversity and related human health impacts, generates considerable negative economic effects.

3. Dose-effect relationships

Dose-effect relationships provide essential data allowing to quantify the public health impact of a particular exposure and are a basis for establishing guidelines.

On fipronil and neonicotinoids specific dose-effect data are limited and insufficiently conclusive. While there is increasing evidence for unintended ecosystem impacts caused by regular concentrations, studies on dose related effects are scarce to inexistent. Also the WIA-assessment, nor the related studies, provide specific information on this topic. Only the Godfrey et al. (2015 a,b) studies make a distinction between lethal and non-lethal effects, a first step up towards a dose-effect analysis. In view however of the complex (health and environment, direct and indirect, long- and short-term) effects which have been identified the issue raises different questions, including:

- What are the effects of exposure at low doses?
- Can a threshold value be proposed?
- What is the shape of the dose-effect curve at low doses exposure?

This advice identifies these major gaps in the current knowledge and advocates more research on the subject.

4. Human health effects

4.1 Introduction

Published data on human health effects of neonicotinoids, a relatively new class of pesticides (Roberts et al., 2012) are scarce. Using neonicotinoids as keyword, this review identified only about 40 publications indexed starting in 2000. Three different conditions have been studied: general population, chronic exposure related to occupational use and acute exposure either accidentally or as a result of suicide attempt. Thus both exposure and effects will be discussed based on those 3 conditions.

4.2 Metabolism

In vitro studies using the human gastro-intestinal cell line Caco-2 showed that imidacloprid is absorbed (Brunet et al., 2004). Imidacloprid is metabolised along two pathways mediated by the liver enzyme cytochrome P_{450} (Schulz-Jander and Casida, 2002):

- by hydroxylation and saturation, this results in the production of hydroxyimidacloprid and an alkene;
- and through the reduction and cutting of a nitroimine reduction resulting in nitrosoimine, guanidine and ureum derivatives.

It is likely that these metabolites are more toxic than imidacloprid by its own (El-Gendy et al., 2010).

4.3 Exposure

Human exposure to pesticides is primarily determined by the amounts introduced in environment. Belgian figures on sales and consumption of neonicotinoids and fipronil are included in section 1 of this advice. This section discusses the internal exposure and its effects.

4.3.1 Biomonitoring

Kavvalakis et al. (2013) compared hair concentrations in rabbit (control vs exposed to imidacloprid) and in people living in rural areas. After 6 months of exposure the rabbits' hair concentrations increased by a factor 60-90x reaching around 40-60 ng/mg. Median hair imidacloprid concentrations in rural residents was 0.03 ng/mg. It was 0.6-1.6 ng/mg in control rabbits. McMahen et al. (2015) found no fipronil in urine of unexposed humans, but fipronil sulfone was detected in serum of 25% of subjects in concentrations ranging 0.1-4 ng/ml. These preliminary data indicate that evidence of exposure can be found in presumably non-exposed subjects.

4.3.2 Occupational

Among 52 occupationally exposed Japanese adults, over 90 % of the individuals showed urinary levels above the limit of detection for imidacloprid, thiamethoxam, clothianidin and dinotefuran, over 50 % for acetamiprid and thiacloprid and 29 % for nitenpyram. The median concentration was the lowest for acetamiprid (0.02 ng/ml), while it was 1.9 and 2.3 ng/ml for imidacloprid and dinotefuran respectively (Ueyama et al., 2014).

Exposure to pesticides, including imidacloprid and its metabolite 6-CNA, was analysed in a group of 135 professional turf applicators in six cities across the United States over three spraying seasons using urinary biomarkers via the collection of 1028 urine samples (Harris et al., 2010).

Twenty-four-hour estimates were calculated and mixed models were applied to describe the variance with respect to city, season, individual, and day of sampling. Imidacloprid showed concentrations that exceeded the detection level in 60 of the 513 24-h samples and 6-CNA in only 5 of the 24-h samples. For imidacloprid, the between-sample variation accounted for the largest percentage of overall variability (approximately 65 %). The large variability between days in the same season observed for imidacloprid suggests the need to take multiple individual samples within a season.

In 159 workers from a factory manufacturing fipronil, Herin et al. (2011) found serum levels of fipronil and its sulfone directly related to the duration of exposure and negatively related to TSH (thyroid-stimulating hormone) suggesting possible central inhibition of TRH (thyrotropin releasing hormone)-TSH, as opposed to increased TSH levels reported in rodents.

4.3.3 Accidental/ Intentional

Taira et al. (2013) evaluated 57 known urinary metabolites of three neonicotinoid pesticides (acetamiprid, imidacloprid, and clothianidin), as well as the parent compounds. Seven metabolites were detected in the urine of 3 subjects suspected of exposure to sub-acute concentrations. Acetamiprid could not be detected in 2 cases and 0.06 ng/ml was measured in the third case. *N*-desmethyl-acetamiprid was determined in the urine of one case, which had been collected on the first visit, at a concentration of 3.2 ng/ml. This study indicates that low or undetectable levels of the original compound in body fluids do not exclude exposure that could be reflected by presence of metabolites.

Mohamed et al. (2009) investigated 68 patients (61 self-ingestions and 7 dermal exposures) exposed to imidacloprid. Median imidacloprid concentration at admission was 10.58 ng/L (range: 0.02-51.25 ng/L). Of the self-poisoning patients, the amount ingested was median 15 mL (IQR 10-50 mL) and the median time to presentation was 4 hours (IQR 2.3-6.0). Most patients only developed mild gastrointestinal symptoms and headache. One patient developed respiratory failure while another was admitted to intensive care due to prolonged sedation. Other studies have confirmed toxic effects after ingestion of 50 mL Imidacloprid (Panigrahi et al., 2009; Viradiya & Mishra, 2011).

Forrester (2014) reported neonicotinoid exposures of 6 Texas poison centers during 2000–2012. 77 % of the 1,142 total exposures contained imidacloprid (77 %). 97 % of the exposures were unintentional, and 97 % occurred at the patient's own residence. The most common routes of exposure were ingestion (51 %), dermal (44 %) and ocular (11 %). The most commonly reported adverse clinical effects included ocular irritation (6 %), dermal irritation (5 %), nausea (3 %), vomiting (2 %), oral irritation (2 %), erythema (2 %) and red eye (2 %).

Besides these studies several cases of lethal poisoning following ingestion of imidacloprid are reported in literature (David et al., 2007; Yeh et al., 2010); insecticide containing 9.7 % imidacloprid (Wu et al., 2001); imidacloprid ingestion leading to blood concentrations post-mortem between 12.5 and 2.05 microg/mL.(Proença et al., 2005); 350 mL imidacloprid (Shadnia & Moghaddam, 2008). These reports point to the sensitivity of the nervous system for imidacloprid.

4.4 Direct and offspring human effects *in vivo*

The only data on effects of neonicotinoids or fipronil in humans *in vivo* have been obtained following accidental or suicidal exposure to high levels.

4.4.1 Occupational/ accidental

Lee et al. (2010) reported data from the Sentinel Event Notification System for Occupational Risks (SENSOR)-Pesticides Program and the California Department of Pesticide Regulation in USA. During a 7-year period, a total of 103 cases of illnesses associated with fipronil exposure were identified in 11 states; ¾ of them resulting from the private use of products and ¼ from work-related use. Neurological complaints (50 %) such as headache, dizziness, and paresthesia, were most frequently mentioned followed by ocular, gastrointestinal and respiratory symptoms or signs. The effects were mild and temporary in 89 % of the cases.

In a comparison between Greek pesticide sprayers vs. non-occupationally exposed rural residents, a by-product of oxidative deoxyribonucleic acid (DNA) damage from blood cells was found to increase in relation to the area and frequency of pesticide application. The DNA damage was more important after using neonicotinoids than other pesticides (Koureas et al., 2014). A Spanish study in pesticide sprayers showed respiratory effects of both short and long term exposure to neonicotinoids associated with reduced pulmonary volumes suggesting restrictive lung disease (Hernandez et al., 2008). In Poland, a 50-year-old male was hospitalised after 5h of spraying his field with a solution of fipronil (Chodorowski and Anand, 2004). The patient complained of headache, nausea, vertigo and weakness. All symptoms resolved spontaneously after about 5 hours. The patient was fully conscious with the blood pressure and heart rate within a normal range. There were no seizures, other neurological deficits, signs of conjunctivitis or skin irritation. Fung et al. (2003) reported a case of accidental ingestion of a commercial household product containing fipronil by a 77-year-old woman who did not develop obvious toxicity signs (mild subjective impairment of sensory effects disappearing spontaneously after half an hour).

Of the 1,142 total neonicotinoid exposures reported to the six Texas poison centres during 2000-2012, 97 % were unintentional and occurred at the patient's own residence (Forrester, 2014). Most products contained imidacloprid (77 %) or dinotefuran (17 %). The exposures were seasonal and half of them were reported during the May-August period. The most common routes were by ingestion (51 %) and dermal (44 %) exposure. Exposure occured more frequently in patients of 20 years or older (61 %) as well as in children younger than 5 years of age (28 %). As compared to two other groups of insecticides (carbamate/chlorinated hydrocarbon/organophosphate and pyrethroid/pyrethrin), the serious medical outcomes were significantly lower for neonicotinoids. Although a few clinical effects might be expected, the majority of neonicotinoid exposures may be managed outside health care facilities with few clinical effects expected. Neonicotinoid insecticides result in less serious outcomes than other major types of insecticides. This has also been observed in a study in the United Kingdom that examined 105 unintentional neonicotinoid exposures reported to poison centres (Adams et al., 2013). However, Agha et al. (2012) consider that fatality and morbidity due to imidacloprid might be underestimated. They report the case of a 62-year-old farmer who sprayed insecticide that contains 30 % imidacloprid on his trees for 30 min almost one week prior to showing up at the emergency department without wearing any mask or gloves. He had a history of fever, disorientation, red-coloured urine, lower abdominal pain and vomiting during four days prior to admission. This is likely the first reported case of leukoclastic vasculitis due to imidacloprid skin contact and inhalation exposure.

4.4.2 Intentional

A retrospective review of 70 cases of imidacloprid poisoning in Taiwan (Phua et al., 2009) showed a cholinergic syndrome that resulted in major effects including coma, and aspiration pneumonia in 8 patients, while two more died. The authors concluded that neonicotinoid insecticides could be safer than older classes of insecticides because they resulted in severe effects less frequently. This finding could be explained by high selectivity of neonicotinoid insecticides for insect nAChRs and high water solubility, which reduces their ability to penetrate the mammalian blood–brain barrier and causing less toxic effects to the central nervous system (CNS). A review of the literature including a study by Mohamed et al. (2009), emphasized that the severity of poisoning was neither proportional to the plasma neonicotinoid concentrations nor related to oral, dermal or inhalation routes of exposure (Lin et al., 2013).

Acute human self-poisoning with fipronil was reported for seven patients in Sri Lanka (Mohamed et al., 2004). Among these, only two showed significant CNS toxicity accompanied by sweating, nausea, vomiting and agitation. Within 12h following the ingestion, all patients were essentially asymptomatic. They were discharged from the hospital within four days after admission. Pharmacokinetic data are available for six patients: the highest concentrations are observed at admission to the hospital; fipronil disappears rapidly from the blood during the first 15-20h; thereafter the fipronil concentration plateaued as a result of its slow elimination and metabolism of sulfone. Therefore the management of these patients should focus on supportive care and early treatment of the symptoms.

4.4.3 Offspring effects

Some individuals are more sensitive to the effects of pesticides/biocides than others. Sensitivity is related to their developmental stage of life, physiology and/or health status. Most at risk are fertile women intending to become pregnant, pregnant women, breast feeding mothers and children from infancy through to adolescence. If exposure to toxicants occurs at critical developmental periods, adverse effects may result. The foetus is particularly vulnerable due to its fast growth, the process of cellular differentiation, the immaturity of its metabolic pathways and the stage of development of vital organs.

Very few data have been published on neonicotinoids exposure and offspring effects. Among the offspring of residents in an agricultural area of California (San Joaquin Valley) where pesticides are used, a statistical significant increased risk of congenital heart disease (Tetralogy of Fallot) was observed after exposure to imidacloprid [adjusted odds ratio (aOR)=2.4; 95% confidence interval (CI): 1.1-5.4; exposed cases, n=9] (Carmichael et al., 2014). The same authors reported a general lack of association between residential pesticide exposure estimates (based on residential proximity to agricultural pesticide application during early pregnancy) and risk of neural tube defects and orofacial clefts among the offspring in the San Joaquin Valley (neonicotinoid and risk of anencephaly: aOR=2.5, 95%CI: 0.9-7.1; n=6. Neonicotinoid and cleft lip with or without cleft palate: aOR=1.4, 95%CI: 0.7-2.7; n=17) (Yang et al., 2014). However, there were relatively few elevated odds ratios with 95% confidence intervals that excluded 1 after adjustment for relevant covariates. Thus, because of the sizable number of the comparisons the association may have emerged by chance.

4.5 Indirect human health effects

Present day human activities may lead to ecosystem deterioration, biodiversity loss and a loss of ecosystem services. This will affect human health in any case in the long run, be it that the impact may vary greatly across the globe (Millennium Ecosystem Assessment, 2005; Romanelli et al., 2015; Whitmee et al 2015). Given the global and abundant use of neonicotinoids and the indications of effects outside the primary targets, *i.e.* other species apart from pests, these substances may be expected to contribute to ecosystem deterioration as well. Consequently effects on human health and well-being following indirect pathways and becoming manifest after years should be a point of attention and of further study.

As a large part of our food supply depends on pollination by bees and other insects, food production and quality may be affected as well by pollinator decline, in which neonicotinoid use, apart from other stressors, may be instrumental. Studies on global food impact give rise to concern (Klein et al., 2007; Eilers et al., 2011; Ellis et al., 2015; Nicole, 2015; Smith et al, 2015). Vitamin A may be a nutritional component particularly affected (Ellis et al.,2015).

4.6 Mechanisms of action

4.6.1 Direct effects in vitro

Hodgson and Rose (2007, 2008) reviewed studies of pesticide effects on human liver microsomes. Among a number of pesticides, fipronil is the most potent inducer of cytochrome P450 isoforms possibly resulting in disturbances of liver metabolism of several compounds including sex steroids. Fipronil inhibits the testosterone metabolism. Effects occur at low concentrations of endogenous substrates. Increased fipronil concentrations cause hepatotoxicity. In a human neuroblastoma cell line, fipronil activates apoptotic processes. In isolated mitochondria, fipronil uncouples oxidative phosphorylation (Vidau et al., 2011). Using human hepatocytes and rodent adipocytes or myotubes, it was shown that 10-20 µM of imidacloprid reduces insulin-induced glucose uptake through altering the intracellular signalling of kinase (Kim et al., 2013). Thiacloprid decreases the mitotic index, the proliferation index and the nuclear division index; it increases chromosome aberrations in cultured human peripheral blood lymphocytes (Kocaman et al., 2014). All together, these *in vitro* data refer to possible mechanisms underlying the fipronil and neonicotonoid toxicity and endocrine disruption in different tissues.

4.6.2 Genotoxicity

The genotoxic potential of imidacloprid, acetamiprid and thiacloprid was merely studied on human peripheral blood lymphocytes. Different genotoxic effects have been investigated: DNA damage has been demonstrated using the comet assay and sister chromatide exchange tests, whereas chromosome mutations have been demonstrated by micronucleus and chromosome aberration tests (Costa et al., 2009; Demsia et al., 2007; Feng et al., 2005; Karabay and Oguz, 2005; Kocaman and Topaktas; 2007; Kocaman et al., 2014; Stivaktakis et al., 2010).

Five studies on imidacloprid are not easily comparable due to the different concentrations, exposure periods, and genotoxic endpoints recorded in each of them (Costa et al., 2009; Demsia et al., 2007; Feng et al., 2005; Karabay and Oguz, 2005; Stivaktakis et al., 2010).

Costa et al. (2009) reported that imidacloprid at concentrations below 20 µM is not genotoxic (comet assay) to human lymphocytes *in vitro* (even following metabolic activation). A significant increase in the micronuclei incidence is observed at 20 µM and is increased slightly by metabolic activation.

The commercial preparation was observed to be slightly more genotoxic than the pure substance. These results are in line with the observations of Feng et al. (2005) showing significant effects at doses ranging between 0.4 µM and 2 µM. Contrarily, Demsia et al. (2007) did not observe any effect. However, they used both micronucleus and sister chromatid exchange tests, and suggest that the controversial results with those of Feng may be explained by the difference in control value between both studies. More recently, Stivaktakis et al. (2010) did not note any effect of imidacloprid at 20 µM. The authors conclude a safety level for imidacloprid exists for human exposure. Karabay and Oguz (2005) observed a synergistic effect of the organophosphate methamidophos and imidacloprid in the formulated products. This causes an increase in the risk for non-target organisms. Their observations are based on a bone marrow chromosome aberration assay, a micronucleus test in Wistar albino rats (50 and 100 mg/kg imidacloprid, 2.5 and 5 mg/kg methamidophos and 2.5 and 5 mg/kg imidacloprid plus methamidophos) and a bacterial mutation assay (*Salmonella*/microsome mutagenicity assay). Dose-related increases in the micronucleus incidence ($P < 0.05$) and with the two *Salmonella* strains (TA98 and TA100) were found. All tested doses of the insecticides showed mutagenic activity in the presence of a metabolising extract (S9 mix).

Calderón-Segura et al. (2012) studied genotoxicity of commercial neonicotinoide insecticides in human peripheral blood lymphocytes using the comet assay, and found that exposure to 9.5×10^{-6} to 5.7×10^{-5} M of the commercial insecticide Jade (imidacloprid); 2.8×10^{-4} to 1.7×10^{-3} M Gaucho (imidacloprid); 0.6×10^{-1} to 1.4×10^{-1} M Calypso (thiacloprid); 1.2×10^{-1} to 9.5×10^{-1} M Poncho (clothianidin) for 2 h induced a significant increase of DNA damage with a concentration-dependent relationship.

Although the studies are not univocal, there is substantial evidence indicating that imidacloprid has genotoxic properties. However, the range of concentrations tested is rather large and a discussion about the pertinence of the dose used in the different assays lacks.

Kocaman et al. (2007, 2014) studied the genotoxic properties of acetamiprid and thiacloprid with a similar protocol including sister chromatid exchanges, chromosomal aberrations and micronucleus tests. Acetamiprid was tested in a narrow range from c.a. 112 to c.a. 180 µM and thiacloprid from c.a. 300 to 1200 µM. Sister chromatid exchange and chromosome aberration tests reveal significant differences with the controls at all concentrations for both compounds. Micronuclei significantly increased at all acetamiprid concentrations but the lowest one (25 µg/mL), while micronuclei formation is systematically increased with thiacloprid in the presence of an exogeneous metabolic activation system.

Acetamiprid and thiacloprid were shown having genotoxic properties *in vitro* in the above mentioned publications of the open scientific literature. As mentioned before, imidacloprid does not show a dose dependent cytogenetic effect.

Complete toxicity packages were submitted at the occasion of the EU-review of all neonicotinoids (imidacloprid, thiamethoxam, clothianidin, thiacloprid and acetamiprid) and fipronil. Particularly their genotoxicity has been fully investigated, including the *in vivo* assays. For all neonicotinoids, it has been concluded that, whereas positive results may occasionally have been observed *in vitro*, none exhibited genotoxic effects *in vivo*. As a matter of fact, a positive finding *in vivo* would have precluded any approval of neonicotinoids and fipronil both under Directive 91/414/EEC and Regulation (EC) No 1107/2009, as any genotoxic finding without threshold would have precluded the establishment of Human Health reference doses and consequently any risk evaluation. The Council notes however that *in vivo* effects are reported in several scientific papers. Bagri et al. (2015) observed sperm head abnormalities in Swiss albino mice at 22, 11 and 5.5 mg/kg/day of imidacloprid for 14 or 28 days. Bagri et al. (2016) observed, for imidacloprid**,** a dose and time dependant increase in micronuclei and

chromosomal aberrations in bone marrow of Swiss albino male mice at doses of 5.5, 11 and 22 mg/kg body weight for 7, 14 and 28 days.
Bhinder et al. (2012) found imidacloprid and thiamethoxam to induce mutations in *Anopheles stephensi*. Zang et al (1999) found imidacloprid to be genotoxic in the earthworm. Lin et al. (2005) found imidacloprid to enhance genotoxicity of cadmium in *Vicia faba* plants. Imidacloprid induced chromosomal alterations and increased the frequency of micronuclei in *Allium cepa* and *Tradescantia pallida* (Rodríguez et al., 2015). Sekeroglu et al. (2013) found thiacloprid to induce an increase in chromosome aberrations in rat bone marrow cells at 22.5 mg/kg/day for 30 days, and after a single dose of 112.5 mg/kg; the 30 day treatment also caused a significant increase in micronucleus formation.

The data in table 5 provide an overview of the genotoxic experiments and are summarised from the EU-Peer-Reviews of imidacloprid, acetamiprid and thiacloprid:

Table 5. Results from the EU peer-reviewed genotoxicity data (extracted from the EU-draft assessment reports, 2004 and 2008)

Imidacloprid

Test system	Indicator cells	Results
Ames bacterial reverse mutation assay (4 studies)	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2uvrA	Negative
Recombination assay	*B. subtilis*	Negative
In vitro mammalian cell gene mutation assay	Chinese hamster ovary cells/HPRT locus	Negative
Mitotic recombination	*S. cerevisiae*	Negative
In vitro unscheduled DNA	Rat liver cells	Negative
Sister chromatid exchange	Chinese hamster ovary cells	Positive with and without S9
Sister chromatid exchange	Chinese hamster ovary cells	Negative
In vitro chromosome aberration assay	Human lymphocytes	Positive with and without S9
In vivo chromosome aberration study	Chinese hamster bone marrow	Negative
In vivo micronucleus assay	Bone marrow polychromatic erythrocytes of NMRI mice	Negative
Sister chromatid exchange	Chinese hamster bone marrow	Negative
In vivo chromosome aberration study	Germ-cells of NMRI mice (spermatogonia)	Negative

*EU-evaluation **Imidacloprid** (EFSA conclusions, see reference list)*

Acetamiprid:

Test system	Indicator cells	Result
Ames bacterial reverse mutationa assay	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2uvrA	Negative
In vitro mammalian cell gene mutation assay	Chinese hamster ovary cells/HPRT locus	Negative
In vitro chromosome aberration assay	Chinese hamster ovary cells	Positive with and without S9
In vitro unscheduled DNA	Rat liver cells	Negative
In vivo mouse micronucleus assay	Bone marrow polychromatic erythrocytes of CD-1 mice	Negative
In vivo chromosome aberration study	Rat bone marrow	Negative
In vivo unscheduled DNA	Rat liver cells	Negative

*EU-evaluation **Acetamiprid** (agreed endpoints, see reference list)*

Thiacloprid

Test system	Indicator cells	Result
Ames bacterial reverse mutation assay	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2uvrA	Negative
In vitro mammalian cell gene mutation assay	Chinese hamster ovary cells/HPRT locus	Negative
In vitro chromosome aberration assay	V79 cells	Negative
In vitro unscheduled DNA	Rat liver cells	Negative
In vivo mouse micronucleus assay	Bone marrow polychromatic erythrocytes of CD-1 mice	Negative

*EU-evaluation **Thiacloprid** (agreed endpoints, see reference list)*

In conclusion: whereas positive genotoxicity results were reported *in vitro* for both acetamiprid and thiacloprid, EU-regulatory studies do not confirm that the compounds would be clastogenic *in vivo*.

4.7 Neurodevelopmental studies of EU-approved neonicotinoids

The SHC was asked, among others, to appraise the possible impact on human health consecutively to an exposure to neonicotinoids authorised in Belgium, with special attention to the opinion of the EFSA scientific Panel on Plant Protection Products and their Residues (PPR) on the developmental neurotoxicity potential of acetamiprid and imidacloprid, and the scientific rationale to revise the toxicological reference values.

Therefore, a short summary of the opinion of the EFSA PPR panel, as well as the key papers cited, is presented and discussed. The proposals are extended to the other neonicotinoids approved until now.

Background:

An *in vitro* study (Kimura-Kuroda et al., 2012) suggests that excitation and/or desensitisation of nicotinic acetylcholine receptors (nAChRs) by acetamiprid and imidacloprid might affect the developing mammalian nervous systems, as demonstrated for nicotine. The Directorate General for Health and Food Safety of the European Commission mandated EFSA to examine the paper, in order to establish:

- if acetamiprid and imidacloprid exhibit developmental neurotoxic effects;
- if acetamiprid and imidacloprid have adequately been assessed until now;
- if the existing Reference Doses (RfD), *i.e.* Acceptable Daily Intake (ADI), Acceptable Operator Exposure Level (AOEL) and Acute Reference Dose (ARfD), are still protective;
- whether *in vitro* systems like those of Kimura-Kuroda et al. should be used in the regulatory studies for neurotoxic compounds of the neonicotinoid class.

4.7.1 The study by Kimura-Kuroda et al. (2012)

The experimental design and the results of the study are described in annex 6.

Discussion:

The authors highlight the importance of nAChRs during development and the adverse effects of nicotine. In the developing brain, α4β2 and α7 subtypes of the nAChR have been implicated in neuronal proliferation, apoptosis, migration, differentiation, synapse formation, and neural-circuit formation; nicotine and neonicotinoids could affect these processes when they activate nAChRs. They also highlight that chronic exposure to nicotine causes a series of adverse effects on the normal development of a child. Perinatal exposure to nicotine is a known risk factor for sudden infant death, low-birth-weight infants and attention deficit hyperactivity disorder (ADHD). Further, nicotine exposure modulates the cell-adhesion and cell-death/survival systems in the brain of adolescent rats and may lead to numerous behavioural and physiological deficits. Since newborn rats are equivalent to the human embryo from the aspect of brain development, the effects of the neonicotinoids on neonatal rat cerebellar cultures imply that there may well be prenatal adverse effects of neonicotinoids in humans.

The PPR panel is somewhat more cautious in its conclusions (EFSA, 2013).

The PPR considers the methodology which is widely used in *in vitro* neurotoxicity (NT) studies, suitable.

Notwithstanding the fact that the PPR panel acknowledges the value of the published *in vitro*-studies, some methodological shortcomings were highlighted. Nevertheless, an attempt was made putting into context the *in vitro* data in connection with the existing *in vivo* studies on the neonicotinoids.

The concentrations which apply *in vitro* to the human toxicity studies and these used in the animal *in vivo* studies were compared.

- Extensive data on the concentration of nicotine in smokers' blood are cited; they vary from 0.067 µM to 0.307 µM.
- Published cases indicated blood concentrations after acetamiprid intoxications vary from 10.7 - 268 µM.
- Plasma concentrations after imidacloprid self-poisoning of 28 confirmed cases mention a median value of 10.58 ng/L (0.047 µM, range: 0.02 - 51.25 ng/L, Interquartile Range (IQR): 3.84 - 15.58 ng/L).

The data in the discussed publication are also linked with extensive literature data in animals. For nicotine, an intrafemoral artery injection of 1 mg/kg Body Weight (BW) resulted in a plasmatic peak of 0.0021 µM (10'). For imidacloprid, oral administration of 1 mg/kg BW and 5 mg/kg BW resulted in plasmatic C_{max} values of respectively 0.72 µg/mL (2.8 µM) and 13 µg/mL (50 µM).

One concluded that, while the tested concentrations for nicotine were overly high when compared to plasma values in humans and animals, the tested concentrations for the neonicotinoids acetamiprid and imidacloprid (1 - 100 µM) are realistic.

4.7.2 Regulatory studies on acetamiprid and imidacloprid

4.7.2.1 Acetamiprid

Whereas a Developmental Neurotoxicity (DNT) study was not available during the EU peer review, the study was made available by the US Environmental Protection Agency (EPA, 2004) and is summarised below.

In a DNT study compliant with the US-EPA guideline OPPTS 870.6300 (August 1998), acetamiprid was administered to 25 mated female rats dosed by gavage at doses of 0, 2.5, 10 and 45 mg/kg/day from gestation day (GD) 6 through lactation day (LD) 21.

The maternal NOAEL was 10 mg/kg per day based on decreased body weight and decreased body weight gain during gestation at 45 mg/kg BW per day. At this high dose level the offspring showed treatment-related decreased body weights and decreased body weight gains in males and females post-weaning, decreased pre-weaning survival (Postnatal Day (PND) 0-1), and decreased maximum auditory startle response in males. The treatment had no adverse effects on clinical signs, developmental landmarks, functional observational battery (FOB), brain weight or brain morphology. However, the reviewer highlights that some subtle effects were reported on brain morphometry at the top-dose (brain width declined in PND 72 males by *ca.* 5 % $p \leq 0.01$, length of the dentate hilus (ventral limb) decreased in PND 72 females by *ca.* 15 % $p \leq 0.05$).

No conclusions could be made on the assessment of the motor activity due to the low confidence in the data because of problems with the control data (*i.e.*, the normal developmental pattern was not seen in control animals).

The maximum auditory startle response amplitude decreased by 27 % (PND 20) and 40 % (PND 60) at 10 mg/kg BW per day, and by 42 % (PND 20) and 53 % (PND 60) at 45 mg/kg BW per day. However, only in the latter case the endpoint was considered as treatment-related by the US-EPA. No conclusion was made on the effects of acetamiprid on learning and memory because of the high variability of the data.

The PPR Panel considers that, notwithstanding the claimed guideline compliance of this study, the data do not allow any firm conclusion since important endpoints such as motor activity, learning and memory evaluation could not be properly assessed. Moreover, insufficient arguments support the straight conclusion of the study reporting that (seemingly dose-related) reduced auditory startle responses in offspring first noted at 10 mg/kg BW were not related to treatment. Overall the study can only provide supportive evidence, but is inadequate for a proper characterisation of the effects and dose-response relationship between acetamiprid and developmental neurotoxicity.

The PPR Panel recommends that, based on these uncertainties and methodological drawbacks, the NOAEL for DNT should be conservatively set at 2.5 mg/kg BW per day (the lowest dose).

4.7.2.2 Imidacloprid

The same study summarised in the imidacloprid Draft Assessment Report (DAR) and compliant to US-EPA guideline OPPTS 870.6300 (August 1998), was also available to US EPA. The average daily intake of imidacloprid (administered to 30 parent female rats from GD 0 through PND 21) was 0, 8, 19.4, 54.7 mg/kg BW/ day during gestation. Observed treatment-related effects for maternal animals include a decrease in food consumption for females

(dams) in the high dose group as compared to the controls during the 3rd week of gestation and the 1st week of lactation.
There was also a decrease in body weight gain during LD d 0-7. The maternal NOAEL was 19.5 mg/kg BW per day taking into account the decreased food consumption and decreased body weight gain during lactation.

Treatment-related effects for offspring were limited to the high dose group. Body weights of high-doses both in males and females significantly decreased by 11-13 % ($p < 0.05$) prior to and after weaning, with recovery (in females back to control levels by PND 50, in males to a 4 % difference that persisted to study termination). Body weight gains were also decreased 12-23 % during lactation, with recovery by PND 17. Overall motor activity decreased on PND 17 in high-dose males (38 %) and females (31 %) and on PND 21 in females (37 %), although the differences were not statistically significant. The effects on motor activity were treatment related because of their magnitude and the occurrence at the high dose in both sexes during the period of exposure. High doses in females at PND11 resulted in a 5.5% decrease in thickness of the *caudate/putamen* in comparison to the controls (2.617 *vs.* 2.769 mm). These females also had a 27.6 % reduction in the thickness of the *corpus callosum* (0.436 *vs.* 0.602 mm). The decrease in the *caudate/putamen* width persisted in high dose female animals at study termination (3.677 *vs.* 3.750 mm, $p<0.05$). The offspring NOAEL was 19.5 mg/kg BW per day based on decreased body weight and body weight gain, and decreased motor activity. The NOAEL for neuropathological findings in females was conservatively estimated to be 5.5 mg/kg BW per day based on the application of an extra 10× safety factor to the LOAEL (Lowest Observed Adverse Effect Level) (54.7 mg/kg BW per day) since neuropathology examination was not performed at lower doses.

PPR further noted that the pathological changes observed in basal ganglia (*caudate* and *putamen*) and in the *corpus callosum* may be associated with controlling the motor function. In particular, *putamen* is connected with the *globus pallidus* and the *substantia nigra* through various nervous pathways. Since the *putamen* is involved in movement regulation and influences various types of learning, a decrease in thickness of this structure could be due to a decreased number of neurons/glia ultimately leading to decreased motor activity. The neuronal nAChRs may be involved in some of this neuropathology, thus a possible link between morphological and functional changes should be taken into account. Since a neuropathological assessment was first performed on PND 11, the timeline of the imidacloprid developmental neurotoxicity could not be determined. Therefore, evidence from the DNT study in rats suggests that imidacloprid may affect the development of the brain structures, although the current data may be insufficient for a proper characterisation of the effects and dose-response relationships of imidacloprid's developmental neurotoxicity during pre- and postnatal periods.

4.7.3 Adaptations of the reference doses (RfDs) for acetamiprid and imidacloprid.

As shown in Table 6, the PPR proposed to reduce the RfDs of acetamiprid to 0.025 mg/kg BW/d, and of imidacloprid (only AOEL and ARfD) to 0.06 mg/kg BW/d.

The SHC agrees that these proposals would be re-examined by the EU.

Table 6. Overview of existing (EU peer-reviewed) and proposed Reference doses (RfDs) of the existing neonicotinoids

A.s.	Type RfD	(mg/kg BW/d)	NOAEL (mg/kg BW/d)	Study relied upon	Revised RfD (mg/kg BW/d)	DNT NOAEL (mg/kg BW/d)	DNT LOAEL (mg/kg BW/d)
Acetamiprid	ADI	0.07	7	2 yr rat / 2G	**0.025**	2.5° (→0.025)	10
	AOEL	0.124	12.4	90d rat	**0.025**		
	ARfD	0.1	10	Acute NT rat	**0.025**		
Imidacloprid	ADI	0.06	6	2 yr rat	0.06 (n.c.)	5.5‡(→0.055)	55
	AOEL	0.08	8	28-90d dog	**0.06**		
	ARfD	0.08	8	90d dog/rabbit development	**0.06**		
Clothianidin	ADI	0.097	9.7	2 yr rat	n.c.	43° (→0.43)	142
	AOEL	0.10	10	Rat/rabbit development	n.c.		
	ARfD	0.10	10	Rat/rabbit development	n.c.		
Thiamethoxam	ADI	0.026	2.6	18 mo mouse	n.c.	34.5* (→0.345)	299 (→0.299)§
	AOEL	0.08	8	90d dog	n.c.		
	ARfD	0.5	50	rabbit development	**0.3** t.b.c.		
Thiacloprid	ADI	0.01	1	2 yr rat	n.c.	4.4** (→0.044)	25.6 (→0.0256) §
	AOEL	0.02	2	rabbit development	n.c.		
	ARfD	0.03	3	Acute NT rat	n.c.		

,: based upon DNT studies evaluated by the US-EPA (2003**, 2005*); further Peer Review is anticipated at renewal of the a.s. in the EU.*
°: real NOAEL's based on developmental neurotoxicity findings at the next-higher dose
‡: an extra uncertainty factor (10×) on the LOAEL was applied by PPR, to cover missing brain thickness measurements at intermediary doses
§: applying the same uncertainty factor (10×) on the LOAEL, and deriving a 'worst-case' RfD to cover missing brain thickness measurements at intermediary doses, indicates a potential slight underestimation of the ARfD for Thiamethoxam, but is sufficiently conservative for Thiacloprid.
n.c.: no change; t.b.c.: to be confirmed
The values labelled by "→" are derived using a 10x10 assessment factor on the NOAEL or a 10x10x10 assessment factor on the LOAEL

4.7.4 Further evaluations of other neonicotinoids

To extend the evaluation to the other neonicotinoids approved in the EU, the SHC checked the existing RfDs of the compounds clothianidin, thiamethoxam and thiacloprid with the available DNT studies (see annex 6).

4.7.5 Conclusions

a. Do acetamiprid and imidacloprid exhibit developmental neurotoxic effects?

Indications exist that acetamiprid and imidacloprid show DNT potential, and may (slightly) affect neural development and function at systemically toxic doses (*i.e.* at doses where other toxicity findings are observed).

b. Have acetamiprid and imidacloprid adequately been assessed until now?

According to the panel, the DNT study on acetamiprid was suboptimal because (i) motor activity and memory could not be adequately assessed, and (ii) uncertainty exists about the auditory startle response in the pups. The acetamiprid and imidacloprid study shows limitations impairing to conclude on behavioural effects and/or a dose-response relationship for the brain morphometry.

c. Are the existing Reference doses (RfDs) still protective?

The ADI, AOEL and ARfD of acetamiprid and the AOEL and ARfD of imidacloprid were tentatively reduced to 0.025 mg/kg BW/d and 0.06 mg/kg BW/d, respectively.

d. Are in vitro systems like those of Kimura-Kuroda et al. useful in the regulatory studies for neurotoxic compounds of the neonicotinoid class?

Current *in vitro* systems cannot substitute for *in vivo* DNT, since (i) only a limited number of neural cell types are assessed, (ii) behavioural outcomes remain not covered *in vitro*. If properly validated, the *in vitro* tests could provide indicators as a first alert and/or to prioritise the further screening of compounds.

DNT studies on neonicotinoids should describe their plausible mode of action (MoA) on the neural system.

Concerning the other neonicotinoids on the EU market (clothianidin, thiamethoxam and thiacloprid), taking into account the lowest relevant DNT endpoint in pups, most existing reference doses are properly covered.

Most compounds tested in the DNT studies act on the brain at the highest doses. Although morphometric measurements are characterized by large variations, and the effects are relatively modest, they are consistent, and often accompanied by slight, but potentially relevant behavioural changes. For acetamiprid and imidacloprid, the possibility exists that the observed effects are associated to both maternal and/or pup systemic toxicity and that they are not *per se* a consequence of neurotoxicity. However, given the plausibility of a neurotoxic effect of neonicotinoids (supported by the cited *in vitro* study), this hypothesis cannot be discarded completely.

Except for clothianidin, the top-dose histometric findings for thiamethoxam and thiacloprid are not or insufficiently assessed at the intermediate doses. Nevertheless, applying a conservative high assessment factor on the LOAELs would lead to RfD's which would not be meaningfully lower than those obtained by the other studies (except perhaps for the ARfD of thiamethoxam). This means that the drivers for both the consumer and non-consumer reference doses are not necessarily the DNT studies.

Overall, while neonicotinoids have been shown to demonstrate characteristics of nicotine-like effects at high dose *in vivo*, consistent with specific vertebrate nAChR agonism, the much lower binding affinity compared with that of nicotine is widely accepted being the principal factor for the lower toxicity of the neonicotinoids. While the cited *in vitro* study would indicate that nicotine and neonicotinoids display comparable physiological/neurotoxic effects, it is still unclear if these data may be extrapolated *in vivo*.
However, a conservative re-appraisal of the existing DNT studies on neonicotinoids indicates that the existing RfD's of clothianidin and thiacloprid (and partly thiamethoxam) are covering potential developmental NT effects. For acetamiprid and imidacloprid, lower RfD's may be proposed.

As for acetamiprid and imidacloprid, the DNT of all neonicotinoids will be re-assessed at the EU-level at the occasion of their renewal under Regulation no 1107/2009. However, since several Member States expressed a request to have a discussion on the basis of the original studies, EU authorities can initiate a procedure to assess these.

The SHC agrees that these proposals would be re-examined at the EU-level.

4.8 Comments, gaps to be filled

Data on the interactive aspects among neonicotinoids and between neonicotinoids and related substances are scarce (see section 2.1.3).

The toxicological effects of low-dose pesticide mixtures on human health are largely unknown, although there are growing concerns about their safety. The combined toxicological effects of two or more components of a pesticide mixture can take one of three forms: independent, dose addition or interaction. Not all mixtures of pesticides with similar chemical structures produce additive effects; thus, if they act on multiple sites their mixtures may produce different toxic effects. The additive approach also fails when evaluating mixtures that involve a secondary chemical that changes the toxicokinetics of the pesticide as a result of its increased activation or decreased detoxification, which is followed by an enhanced or reduced toxicity, respectively (Hernandez et al., 2013).

These (and other) gaps provide significant uncertainty on the impacts of the environmental health problems. Therefore a precautionary attitude is indicated when establishing standards.

Evidence based information indicates that thiacloprid, which is used both as an insecticidal plant protection product and a biocidal product active for use in wood preservatives, induces tumours. The European Chemicals Agency (ECHA) (ECHA, 2015) agreed to classify thiacloprid as toxic if swallowed (Acute Tox. 3; H301) and harmful if inhaled (Acute Tox. 4; H332), and which may cause drowsiness or dizziness (STOT SE 3; H336). The substance is suspected of causing cancer (Carc. 2; H351), and may damage fertility and the unborn child (Repr. 1B, H360FD), possibly via an endocrine mode of action. Therefore, the substance will be phased out, because 1A or 1B reprotoxicants or endocrine disrupters cannot be approved under the pesticide regulation (Regulation (EC) No 1107/2009). However a more preventive attitude points to, among others, the chemical similarity between the neonicotinoids and the limited sensitivity of the epidemiological approach, to advocate a precautionary attitude towards all neonicotinoids. A molecular-epidemiological approach studying the association between internal exposure to neonicotinoids and biological effects in humans, might contribute importantly to improve knowledge.

IV. CONCLUSIONS ET RECOMMANDATIONS

Dans le présent chapitre, le CSS répond aux questions des ministres et du secrétaire d'État concernant les impacts de l'utilisation des néonicotinoïdes et du fipronil. Le Conseil formulera ensuite plusieurs recommandations générales à l'égard de l'utilisation de ces substances.

La demande à l'origine de cet avis a été suscitée par la publication de l'« Evaluation Mondiale Intégrée sur les risques des néonicotinoïdes et du fipronil pour la diversité et le fonctionnement des écosystèmes » du groupe de travail international sur les pesticides systémiques, appelée « WIA » (Bonmatin et coll., 2014 ; Chagnon et coll., 2014 ; Furlan et Kreutzweiser, 2014 ; Gibbons et coll., 2014 ; Pisa et coll., 2014 ; Simon-Delso et coll., 2014 ; van der Sluijs et coll., 2014). Lors de la préparation du présent rapport, l'European Academies Science Advisory Council a réalisé une étude similaire (appelée « étude de l'EASAC ») (European Academies Science Advisory Council 2015). Le Conseil a par ailleurs pris note des analyses de récentes études scientifiques (Godfray et coll., 2014 ; Godfray et coll., 2015). En répondant aux questions qui sous-tendent le présent avis, le CSS a également tenu compte de ces études récentes.

1. Questions

Évaluation des études du groupe de travail sur les pesticides systémiques publiées dans la revue *Environmental Science and Pollution Research*, portant plus particulièrement sur les éléments suivants :

- La qualité scientifique de la méthodologie utilisée par les auteurs

Évoquant la mise en place du groupe de travail sur les pesticides systémiques, le Congrès de l'Union internationale pour la conservation de la nature a qualifié la WIA d'« évaluation scientifique complète » (IUCN, 2012). Cette étude s'apparente aux rapports établis par les comités scientifiques d'organisations nationales et internationales telles que le CSS. La qualité de ces rapports d'étude dépend de l'exhaustivité des publications étudiées, de l'indépendance et de la composition multidisciplinaire du comité et, le cas échéant, de la diversité des écoles de pensée scientifiques représentées dans le comité. Le CSS ne dispose pas de ressources suffisantes pour procéder à une évaluation approfondie de tous ces aspects.

Concernant les publications étudiées, le Conseil constate que la stratégie adoptée à l'égard de la recherche bibliographique n'est pas clairement précisée dans tous les chapitres (c'est-à-dire les différents articles de la revue, à l'exception de l'article de conclusion (van der Sluijs et coll., 2014). Seul le chapitre relatif aux effets sur les espèces sauvages de vertébrés (Gibbons et coll., 2014) fournit davantage de détails sur la stratégie de compilation de textes. Le Conseil considère que le manque d'information sur la stratégie de compilation de textes constitue une omission. Rien ne lui permet cependant de douter de l'objectivité de la sélection des publications. La WIA (2014) propose une synthèse de 1121 études publiées dans des revues à comité de lecture portant principalement sur les cinq dernières années. Les données analysées proviennent de publications scientifiques accessibles, ce qui constitue une procédure acceptable. Les informations contenues dans les documents réglementaires fournis par le secteur, généralement sous la forme d'un seul résumé, ont été en partie et indirectement prises en compte par l'intermédiaire d'articles.

La WIA n'est ni une « revue systématique » ni une « méta-analyse » (et ne prétend pas l'être), du moins si l'on considère la définition de ces notions donnée par Cochrane, par exemple.[4] [5]

La méthodologie et, par conséquent, les conclusions qui en découlent ne reposent donc pas sur ce qui est généralement perçu comme la rigueur scientifique voulue dans le milieu professionnel médical ou de la santé publique. Cela ne signifie pas pour autant que l'approche utilisée et les conclusions formulées sont incorrectes, non pertinentes ou inutiles. Le Conseil a de bonnes raisons de croire que les résultats de la WIA sont fiables. L'une de ces raisons est la qualité de l'étude, qui se mesure aussi bien à la diversité des publications analysées et au sérieux de l'approche analytique qu'à l'excellente communication des résultats. Une autre raison tient à la concordance des conclusions de l'étude de l'EASAC, réalisée par un comité complètement différent (European Academies Science Advisory Council, 2015), avec ceux de la WIA.

- Les critères retenus pour sélectionner les études analysées, et notamment ceux relatifs à leur pertinence et à leur fiabilité, par référence au document d'orientation de l'EFSA intitulé « Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 » (http://www.efsa.europa.eu/en/efsajournal/pub/2092.htm)

Le document d'orientation de l'EFSA évoqué dans cette question (European Food Safety Authority, 2011) pose comme principe fondamental les revues systématiques définies dans la note de bas de page qui accompagne la réponse à la précédente question (note de bas de page n° 4). La WIA ne s'entendant pas, selon cette définition, comme une revue systématique, les critères visés ne s'appliquent pas dans toute leur rigueur. Cependant, comme précédemment indiqué, l'apport de plus amples informations à l'égard de la sélection des documents aurait amélioré la WIA.

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4 Une revue systématique consiste à rassembler toutes les données empiriques qui répondent à des critères d'éligibilité prédéfinis afin de répondre à une question de recherche spécifique. Elle utilise des méthodes clairement définies et systématiques sélectionnées de façon à minimiser la subjectivité afin d'obtenir des résultats plus fiables à partir desquels des conclusions peuvent être tirées et des décisions prises [...]. Les principales caractéristiques d'une revue systématique sont les suivantes :
- une série d'objectifs précis et des critères d'éligibilité prédéfinis pour les études ;
- une méthodologie clairement définie et reproductible ;
- une recherche systématique visant à identifier toutes les études susceptibles de satisfaire les critères d'éligibilité ;
- une évaluation de la validité des résultats des études prises en compte à travers une évaluation des risques de subjectivité, par exemple : et
- une présentation systématique et une synthèse des caractéristiques et des résultats des études prises en compte.
De nombreuses revues systématiques incluent des méta-analyses. Une méta-analyse consiste à utiliser des méthodes statistiques pour résumer les résultats d'études indépendantes [...]. Grâce à la combinaison des informations tirées de toutes les études pertinentes, les méta-analyses peuvent fournir des estimations plus précises des effets sur la santé que celles reposant sur les études isolées incluses dans une revue [...]. Elles facilitent également la recherche de cohérence des données, mais aussi de différences, entre les études (Cochrane Collaboration, 2011, section 1.2).

5 Le CSS constate que dans certains articles de presse, la WIA a parfois été qualifiée de « revue systématique », voire de « méta-analyse ».

En raison des méthodes de publications utilisées – sous la forme d'une série d'articles rédigés par des membres du groupe de travail –, le rapport d'étude de la WIA (l'ensemble des articles) est moins cohérent que s'il avait été structuré en chapitres et comporté, au besoin, des annexes. Le rapport aurait également gagné en clarté si l'approche dite de la chaîne des impacts (population d'organismes, exposition, élément de comparaison et résultat) avait été appliquée ; elle aurait facilité l'interprétation des résultats concernant la causalité. Les conclusions (van der Sluijs et coll., 2014) sont conformes à l'opinion consensuelle des 30 membres du groupe de travail qui s'appuie sur l'analyse de plusieurs articles d'actualité.

- Les doses auxquelles les organismes de test ont été exposés dans les études examinées par le groupe de travail (et en particulier si l'ordre de grandeur de ces doses est similaire à celui des doses auxquelles les organismes sont susceptibles d'être exposés étant donné les applications permises en Belgique)

Les auteurs de la WIA tirent leurs conclusions d'une série d'études très diverses, et notamment de rapports d'expériences en laboratoire, d'observations sur le terrain et d'expériences sur le terrain. Ayant considéré dans leur ensemble les données compilées et analysées, ils affirment que leurs conclusions sont conformes à la situation actuelle sur le terrain. Les auteurs passent également en revue les données incertaines et indiquent que les études menées sur le terrain, en particulier, ne conduisent pas toujours à des résultats cohérents, ce qui est aussi la conclusion d'autres arbitres scientifiques (par ex. Godfray et coll., 2014).

La conformité à la réalité est préoccupante dans une série d'études publiées après la WIA (Williams et coll., 2015 ; Stanley et Raine, 2016). Ces articles plus récents corroborent l'affirmation ci-dessus concernant la « situation actuelle sur le terrain ».

- Si l'ordre de grandeur des doses est similaire, l'impact possible sur la biodiversité en Belgique

Les concentrations de pesticides sur le terrain varient d'un pays à l'autre et d'une région à l'autre. Elles dépendent notamment des pratiques agricoles, du type de culture et des exigences réglementaires. Néanmoins, étant donné la diversité des sources sur lesquelles s'appuient les conclusions de la WIA, celles-ci sont, de manière générale, également valables pour la Belgique, et les préoccupations qui émergent de ces conclusions (voir ci-dessous) concernent la politique de la Belgique en matière d'agriculture et de pesticides (voir par ex. *Vlaamse Milieumaatschappij*, 2015). On observe néanmoins que la quantité de résidus générés par les insecticides en question dans les produits alimentaires demeure relativement faible au vu des connaissances actuelles sur les effets toxiques pour les êtres humains exprimés sous la forme de limites maximales de résidus (AFSCA, 2014 ; AFSCA, 2015).

- Mesures possibles de réduction des risques à élaborer comme des conditions déterminant les autorisations afin de limiter l'exposition des organismes non ciblés à des niveaux acceptables

Cette question laisse entendre qu'à l'égard des autorisations relatives aux insecticides (ou, plus généralement, aux pesticides), le critère le plus pertinent porte sur la limite d'exposition pour les organismes non ciblés. Selon le CSS, une approche ne doit pas être uniquement fondée sur une évaluation substance par substance ; elle doit aussi tenir compte de la santé publique. En réalité, les politiques actuellement en vigueur en Europe et en Belgique en matière de pesticides reposent

sur une approche plus large qualifiée de lutte intégrée contre les ennemis des cultures. Dans ce cadre, les pesticides chimiques ne doivent être utilisés dans l'agriculture qu'en dernier recours. Le Conseil précise ci-après sa pensée sur cette question.

- L'impact possible sur la santé humaine d'une exposition résultant des applications permises en Belgique, en accordant une attention particulière à l'avis scientifique de l'EFSA sur le lien potentiel entre la neurotoxicité développementale et deux néonicotinoïdes – l'acétamipride et l'imidaclopride – et au fondement scientifique de la proposition mentionnée dans cet avis de modifier les valeurs de référence toxicologiques.

D'après les conclusions de la WIA et de l'étude de l'EASAC, des indications montrent que les substances en question ont des effets négatifs sur des organismes non ciblés, et notamment des organismes vertébrés, et ce, au degré de concentration dans l'environnement actuellement autorisé. S'il est vrai que les résultats d'observation et d'expérience ne concernaient pas les êtres humains (la WIA ne tient pas compte des effets sur la santé humaine et l'étude de l'EASAC évoque uniquement les effets sur les services écosystémiques en général), ses conclusions avertissent que les êtres humains sont susceptibles d'être touchés. L'utilisation croissante à une échelle mondiale dans le domaine de l'agriculture risque de déboucher sur des expositions chroniques, y compris, selon les données actuellement disponibles, à des niveaux relativement bas. Cependant, les effets de ces expositions chroniques sont encore mal connus, d'autant que les tests réglementaires actuels ne couvrent pas certaines situations, ni les effets sublétaux sur les organismes de test.

Le CSS a examiné l'avis de l'EFSA concernant l'acétamipride et l'imidaclopride (Groupe scientifique de l'EFSA sur les produits phytopharmaceutiques et leurs résidus, 2013). Le Conseil appuie le raisonnement et les conclusions de ce rapport de l'EFSA, et notamment la modification des valeurs de référence toxicologiques attribuées à ces substances. Il est par ailleurs favorable à l'évaluation d'autres néonicotinoïdes. Néanmoins, ce fait et les conclusions de la WIA et de l'étude de l'EASAC incitent à user de précaution, ce qui suppose des conséquences pour les mesures politiques. Le Conseil précise ci-après sa pensée sur cette question.

2. Champ élargi

Le débat sur les néonicotinoïdes et les autres pesticides systémiques a été suscité par les préoccupations liées au déclin de la population des insectes pollinisateurs, et en particulier des abeilles à miel (Henry et coll., 2012). La WIA et l'étude de l'EASAC ont adopté une perspective plus large et rassemblé, puis évalué les informations relatives aux effets sur des organismes (vertébrés et invertébrés) non ciblés ainsi que sur les services écosystémiques et la santé des écosystèmes. Ces substances peuvent aussi affecter directement et indirectement la santé humaine. Les possibles effets indirects sur la santé (causés par l'impact sur les services systémiques par exemple) ne sont pas pris en compte au moment de la délivrance d'autorisations afférentes à l'utilisation des pesticides.

Le CSS se penche dans cette section sur l'utilisation des néonicotinoïdes et des autres pesticides systémiques.

2.1 Utilisation agricole des néonicotinoïdes

Le cadre d'action qui régit l'utilisation des pesticides dans l'Union européenne est précisé dans la directive sur l'utilisation durable des pesticides (EU, 2009). Cette directive définit et promeut la lutte intégrée contre les ennemis des cultures. L'approche de la lutte intégrée en matière de production agricole vise à réduire l'utilisation de pesticides en faveur de méthodes non chimiques pour la lutte contre les ennemis des cultures. Les produits chimiques sont considérés comme un outil à utiliser en dernier recours. Cette directive constitue actuellement la base de l'utilisation des pesticides à l'échelle fédérale et régionale en Belgique (cf. Brussel, 2013 ; KB, 2013 ; Vlaamse Regering, 2013 ; Wallonie, 2013).

Les pesticides néonicotinoïdes sont massivement utilisés comme enrobage de semences (Jeschke et coll., 2011). Comme l'indiquent dans leurs conclusions la WIA et l'étude de l'EASAC, ce type d'utilisation ne vient pas s'ajouter aux méthodes non chimiques de lutte contre les ennemis des cultures ; en réalité, il remplace complètement ou en grande partie les solutions de substitution non chimiques. Dans le cadre d'action actuel de l'Union européenne et de la Belgique pour la lutte contre les ennemis des cultures, ces utilisations en faveur de la lutte contre les ennemis des cultures reposent sur une conclusion de principe selon laquelle les solutions de substitution non chimiques échouent ou ne sont pas applicables. Il ne relève pas du champ d'études du présent rapport ni de la mission du CSS de commenter cette conclusion de façon détaillée. Le Conseil demande néanmoins aux responsables des politiques en matière de pesticides de prendre note du conflit entre l'utilisation des néonicotinoïdes comme enrobage de semence et la lutte intégrée contre les ennemis des cultures.

Le CSS recommande donc de recadrer le débat sur les insecticides systémiques. La première question à se poser n'est pas de savoir si les néonicotinoïdes offrent une innocuité suffisante pour les abeilles ou d'autres espèces, y compris les êtres humains. Il convient en premier lieu de se demander s'il existe d'autres méthodes de protection des plantes qui n'utiliseraient pas de produits chimiques, présenteraient moins de danger pour l'environnement et la santé humaine, et seraient économiquement réalisables, voire plus profitables.

S'il était possible de répondre positivement à cette question, ces insecticides ne seraient plus un des facteurs concourant au déclin de la population d'abeilles, ni un danger pour d'autres espèces, car leur utilisation cesserait progressivement. Si les méthodes alternatives de protection des plantes s'avèrent plus difficilement réalisables ou moins profitables sur le plan économique, il convient de procéder à une évaluation appropriée des risques qu'implique l'utilisation d'insecticides, en tenant compte des potentiels aspects économiques, mais aussi d'autres aspects pertinents, tels que les effets sur la santé humaine. Le CSS donne son opinion à l'égard des aspects relatifs à la santé humaine, mais ne peut que formuler des recommandations procédurales concernant la façon d'établir un équilibre entre les aspects économiques et les autres aspects pertinents sur le plan sociétal, tels que la santé humaine. En guise de recommandation procédurale, le CSS conseille d'organiser une consultation des parties prenantes similaire à celle qui s'est tenue pour interpréter les résultats de biosurveillance humaine en Flandre (Keune et coll., 2009a).[6]

2.2 Utilisations non agricoles

Bien que l'utilisation des néonicotinoïdes ne se cantonne pas uniquement au secteur de l'agriculture, les données relatives à leur utilisation dans les secteurs de l'horticulture, de la sylviculture et de la végétalisation urbaine, par exemple, sont rares (Simon-Delso et coll., 2014). Les utilisations les plus importantes concernant le secteur de l'agriculture, le CSS n'examinera pas d'autres types d'utilisation. Cependant, à l'égard de ces derniers et, en particulier, de l'utilisation par les consommateurs privés et les gestionnaires de territoires, des considérations d'ordre politique similaires à celles exposées dans la précédente section s'appliquent.

2.3 Effets sur la santé humaine

Effets d'une exposition directe

Les données disponibles amènent le CSS à conclure qu'une exposition aux néonicotinoïdes a sur la santé des effets neurotoxiques, perturbateurs pour le système endocrinien, génotoxiques (impliquant un risque de cancer) et, pour le thiaclopride, cancérigènes ; ces effets dépendent des pesticides auxquels sont exposés les individus et du type d'exposition. La vie fœtale et la prime enfance sont des périodes d'exposition critiques.

En outre, en tenant compte d'une marge d'incertitude acceptable, les effets toxiques listés sont sérieux et peuvent être durables. Ces effets se produisent à des niveaux d'exposition actuellement considérés comme faibles. Les niveaux d'exposition actuels en Belgique s'avèrent être inférieurs aux niveaux de référence internationaux (AFSCA, 2014 ; AFSCA, 2015). Étant donné que plusieurs de ces niveaux de référence sont en cours de réexamen par l'UE ou seront réexaminés au cours des prochaines années, la prudence s'impose[7].

6 Pour de plus amples informations sur la consultation des parties prenantes, le CSS s'est référé à Elliott et coll., 2005 ; Hage et Leroy, 2008.

7 D'après les informations du CSS, les néonicotinoïdes et le fipronil feront prochainement l'objet d'un réexamen dans le cadre du Règlement (CE) n° 1107/2009 (renouvellement de l'approbation).

Effets dus à la dégradation des écosystèmes et des services écosystémiques

Le CSS attire également l'attention sur les effets indirects sur la santé liés à la dégradation des écosystèmes et des services écosystémiques (European Academies Science Advisory Council, 2015). Un rapport complet sur la qualité et la santé des écosystèmes a été publié au titre de l'Évaluation des écosystèmes pour le millénaire (Millennium Ecosystem Assessment, 2005) et, plus récemment, grâce au travail collaboratif de la Convention sur la diversité biologique et de l'Organisation mondiale de la Santé (Romanelli et coll., 2015) et à une commission de la Fondation Rockefeller et de la revue The Lancet (Whitmee et coll., 2015). Compte tenu de leur utilisation massive à l'échelle mondiale et des signes de leurs effets sur des cibles autres que les cibles principales (c'est-à-dire sur des espèces autres que celles ennemies des cultures) et, par conséquent, sur la qualité des écosystèmes, mais aussi, suivant des voies indirectes et après plusieurs années, sur la santé humaine et le bien-être, les néonicotinoïdes doivent faire l'objet d'une attention particulière et de nouvelles études. À cet égard, le Conseil recommande la mise en place d'une stratégie préventive similaire à celle précédemment évoquée (voir l'encadré 6).

Les effets indirects sur la santé humaine sont largement liés à la production alimentaire (Klein et coll., 2007 ; Eilers et coll., 2011 ; Smith et coll., 2015 ; Ellis et coll., 2015 ; Nicole, 2015). Une grande partie de nos disponibilités alimentaires dépend de la pollinisation assurée par les abeilles et d'autres insectes. Si les néonicotinoïdes et d'autres pesticides similaires portent atteinte à l'abondance des pollinisateurs, la disponibilité des produits alimentaires essentiels risque de se réduire, en particulier dans les pays en développement. Il est nécessaire de réaliser de nouvelles études sur la nature et l'ampleur de ces effets, qui semblent d'ores et déjà affecter une composante nutritionnelle, à savoir la vitamine A (Ellis et coll., 2015).

3. Recommandations générales concernant l'évaluation de l'impact des néonicotinoïdes

Une évaluation scientifique constitue seulement une étape dans le processus de décision de principe. Le rôle du Conseil est de fournir une évaluation scientifique à l'égard des impacts sur la santé, en s'abstenant autant que possible de donner une interprétation politique de son évaluation. Le Conseil doit par ailleurs faire preuve de transparence concernant les principes qui sous-tendent son évaluation, et notamment le principe de précaution (Elliott et Resnik, 2014). Cet aspect est d'autant plus important lorsque la science ne permet pas d'obtenir de résultats suffisamment probants en raison d'incertitudes et de lacunes scientifiques, d'inexactitudes scientifiques ou de divergences d'opinions entre experts.

Dans le cas des néonicotinoïdes, les connaissances relatives à leurs impacts sur la santé des personnes et des écosystèmes sont très lacunaires, ce que montrent la WIA (van der Sluijs et coll., 2014) et l'étude de l'European Academies (European Academies Science Advisory Council, 2015). Ceci est d'autant plus vrai lorsque différents groupes d'acteurs défendent des intérêts différents pouvant les amener à manifester différentes préférences au moment de l'interprétation des informations scientifiques, ce qui ouvre à différentes possibilités d'action. Le CSS recommande donc l'organisation d'une consultation des parties prenantes portant sur les résultats d'une évaluation scientifique (Elliott et coll., 2006 ; Hage et Leroy, 2008 ; Keune et coll., 2009a ; Keune et coll., 2009b). Cette consultation pourrait déboucher sur la définition de critères de décision sociétale et politique qui tiendraient compte de la façon dont les parties prenantes mettent en regard les informations scientifiques recueillies et les possibilités d'action. Les critères possibles pourraient concerner les aspects relatifs à la santé humaine (aussi bien à court qu'à long terme, impliquant des rapports directs et indirects), les aspects écologiques et les aspects économiques liés à l'application des différentes options de lutte contre les ennemis des cultures. Cela constituera le fondement d'un processus décisionnel impartial, éclairé, structuré et transparent (Keune et coll.,2009b).

Le Conseil réaffirme que si les néonicotinoïdes venaient à être considérés, dans des circonstances bien définies, comme un outil de lutte contre les ennemis des cultures à utiliser en dernier recours dans le cadre d'une approche de lutte intégrée contre les ennemis des cultures, la WIA et l'étude de l'EASAC rendent toutes deux compte de motifs d'inquiétude et recommandent l'adoption d'une stratégie préventive. À cet égard, il convient de prendre conscience qu'à l'instar de nombreux autres pesticides, les néonicotinoïdes provoquent une résistance des organismes ciblés. Cela contribue au phénomène suivant : à long terme, les pesticides devront se diversifier de plus en plus et, souvent, devenir plus puissants – ce qui entraînera davantage de dommages environnementaux et de risques pour la santé – pour assurer un rendement agricole (décroissant) (hypothèse dite de la reine rouge (Van Valen, 1973)).

4. Recommandations concernant la réalisation de nouvelles études

Comme nous l'avons vu, les informations relatives à l'exposition de l'environnement et des êtres humains aux néonicotinoïdes, ainsi que leurs possibles effets à court et long terme, sont lacunaires. Les néonicotinoïdes constituent un (nouvel) exemple d'innovation en matière de pesticide, d'agriculture et de technologie dans l'optique de bénéfices qui ne tient pas vraiment compte des connaissances relatives aux possibles effets sur la santé humaine et environnementale (von Gleich, 1999). Le manque de connaissances à l'égard des bénéfices et des dommages possibles est particulièrement grave dans le cas présent, car l'utilisation d'insecticides systémiques augmente rapidement et s'étend au monde entier.

Les nombreuses lacunes identifiées concernant les effets sur la biodiversité et la santé des écosystèmes justifient la réalisation urgente de nouvelles études. Ces questions se situant à la limite du champ d'études du Conseil, ce dernier n'examine aucun thème de recherche plus précis. Il en va de même pour la réalisation indispensable d'une étude sur les autres pratiques agricoles possibles et la participation des parties prenantes au développement de pratiques alternatives.

Les thèmes de recherche suivants concernent les effets sur la santé humaine.

Exposition et concentrations dans l'environnement, effets systémiques et principes directeurs

Les données relatives aux concentrations dans l'environnement sont rares ou peu satisfaisantes. Le Conseil suggère que les informations sur les concentrations d'insecticides dans le sol, les organismes non ciblés et l'eau de surface fassent l'objet d'un contrôle systématique en Belgique. Les effets sur les composantes des écosystèmes doivent également être étudiés et utilisés pour établir des normes écotoxicologiques. Ces données sont aussi pertinentes pour la santé humaine étant donné que les voies d'exposition des êtres humains partent de l'environnement.

Exposition externe et interne des êtres humains

Les êtres humains sont principalement exposés à travers les résidus d'insecticides générés dans les aliments et l'eau de consommation par les pratiques agricoles. Les individus qui résident dans des régions agricoles sont également susceptibles d'être exposés en cas d'applications foliaires. Les éventuelles expositions professionnelles concernent les travailleurs agricoles et les travailleurs des secteurs de la production et de la mise au point de pesticides. Le Conseil recommande la réalisation d'études sur ces expositions, à l'aide non seulement de modèles d'exposition basés sur les concentrations dans l'environnement et le comportement humain, mais aussi de techniques de biosurveillance humaine. Une attention particulière doit être accordée aux insecticides ou à leurs métabolites, susceptibles de traverser le placenta et donc d'entraîner une exposition fœtale.

Enfin, et ce n'est pas négligeable, le Conseil recommande la collecte de données sur les expositions à des mélanges de pesticides, voire à d'autres polluants environnementaux, car les données à cet égard sont, à l'heure actuelle, peu satisfaisantes[8].

Effets sur la santé humaine

Les données relatives aux effets sur la santé humaine sont peu satisfaisantes, en dehors de quelques rares études de cas sur des expositions accidentelles et délibérées. Néanmoins, les données disponibles rendant compte d'effets neurotoxiques à faible niveau, d'effets cancérigènes pour le thiaclopride, d'effets génotoxiques et d'effets perturbateurs pour le système endocrinien, le Conseil recommande la réalisation d'une nouvelle évaluation toxicologique de ces effets.

Les effets d'une exposition chronique à de faibles niveaux de néonicotinoïdes doivent également être examinés. Les informations sur ces effets sont certes lacunaires, mais de plus en plus d'éléments montrent que même si les niveaux d'exposition actuels à ces insecticides n'engendrent pas de graves effets toxiques, ils sont susceptibles de perturber le système endocrinien ou de provoquer d'autres effets à long terme. Des effets génotoxiques ayant été observés in vitro, dans des cellules humaines et à l'aide de tests in vivo sur des animaux, il n'est pas à exclure que les néonicotinoïdes augmentent le risque de cancer. Le CSS conseille d'envisager la réalisation de recherches épidémiologiques, mais suppose que ce type d'étude ne peut être effectué qu'à grande échelle, et donc au niveau international. Il serait par exemple pertinent de mener une étude épidémiologique sur l'effet d'une exposition dans l'utérus. Compte tenu de la propagation relativement récente des insecticides néonicotinoïdes, ce type d'étude doit être conçu de façon longitudinale.

8 Ces données pourraient soutenir les initiatives prises par l'EFSA pour présenter une évaluation cumulative des mélanges de pesticides (par ex., Groupe scientifique de l'EFSA sur les produits phytopharmaceutiques et leurs résidus, 2013).

5. En résumé

En résumé, le CSS est parvenu à la conclusion que la WIA, l'étude de l'EASAC et les autres données de recherche prises en compte fournissent une base suffisante en faveur de l'application d'une stratégie préventive concernant l'utilisation des néonicotinoïdes et des autres pesticides systémiques. Une telle stratégie est nécessaire pour protéger la santé des personnes et des écosystèmes à long terme et doit être considérée comme une nouvelle étape dans l'évolution vers une société plus durable. Dans le domaine de l'agriculture, cette stratégie suppose une application plus stricte de la politique en vigueur concernant la lutte intégrée contre les ennemis des cultures. Cela implique notamment une réévaluation de l'utilisation des substances en question comme enrobage de semence, celle-ci étant désormais privilégiée et non choisie en dernier recours. Cette stratégie nécessite également la réalisation de nouvelles études consacrées aux effets directs sur les organismes biologiques, et notamment les êtres humains. Compte tenu des modes d'action neurologiques et des potentiels effets sur le système endocrinien, génotoxiques ou autres effets à long terme identifiés, le CSS recommande de réévaluer l'adéquation des niveaux de référence toxicologiques actuels. L'utilisation de pesticides systémiques relevant d'un phénomène mondial, cette stratégie doit s'appliquer dans un contexte européen et international. Le CSS conseille au gouvernement belge d'entreprendre des initiatives au niveau international pour promouvoir la stratégie conseillée. Le gouvernement doit, en collaboration avec les autorités régionales, consulter les parties prenantes et les associer à l'élaboration de mesures de précaution appropriées.

Le débat sur les néonicotinoïdes et les autres pesticides systémiques s'inscrit dans un débat plus large sur l'utilisation des pesticides dans le secteur de l'agriculture et leurs autres applications dans l'environnement quotidien. Si l'enjeu de ce débat est clairement européen, et même mondial, il n'en est pas moins pertinent à l'échelle de la Belgique et à une échelle régionale. Les éléments en faveur d'une approche préventive ne sont pas rares au fil de l'Histoire, en particulier en cas d'utilisation massive de produits chimiques malgré un manque de connaissances sur les effets d'une exposition chronique des êtres humains et de l'environnement. En définitive, le Conseil recommande, d'une part, d'accroître l'attention accordée aux méthodes non chimiques de lutte contre les ennemis des cultures et, d'autre part, d'étudier les « points chauds » de l'utilisation des pesticides en Belgique et de les cibler en vue de stratégies préventives.

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VI. APPENDICES

Annex 1. General information and Chemical and Environmental properties of fipronil and neonicotinoids

Pesticide Manual, Footprint database, EU authorization dossiers

Active substance	General information and Chemical properties
Fipronil	**Pesticide type:** Insecticide, Veterinary substance **Substance group:** Phenylpyrazole **Substance origin:** Synthetic **Mode of action:** Broad-spectrum with contact and stomach action. GABA-gated chloride channel antagonist. **Mol. wt.** 437.2 **M.f.** $C_{12}H_4Cl_2F_6N_4OS$ **Form** White solid (powder) **M.p.** 200-201 °C; (tech., 195.5-203 °C) **V.p.** 3.7 x 10^{-4} mPa (25 °C) **KOW** logP = 4.0 (shake flask method) **Henry** 3.7 x 10^{-5} Pa m^3 mol^{-1} (calc.) **S.g./density** 1.477-1.626 (20 °C) **Solubility** In water 1.9 (pH 5), 2.4 (pH 9), 1.9 (distilled) (all in mg/l, 20 °C). In acetone 545.9, dichloromethane 22.3, hexane 0.028, toluene 3.0 (all in g/l, 20 °C). **Stability** Stable in water at pH 5 and 7; slowly hydrolysed at pH 9 (DT_{50} c. 28 d). Stable to heat. Slowly degrades in sunlight (c. 3% loss after 12 d continuous irradiation); rapidly photolysed in aqueous solution (DT_{50} c. 0.33 d). In plants, animals and the environment, fipronil is metabolised via reduction to the sulfide, oxidation to the sulfone, and hydrolysis to the amide. In the presence of sunlight, a photodegradate also forms via sulfoxide extrusion. The sulfide, sulfone and photodegradate are known to act at the GABA receptor site, whereas the amide does not. **Animals** In rats, once absorbed, the distribution and metabolism of fipronil is rapid. Elimination is mainly via the faeces as fipronil and its sulfone. The two major urinary metabolites were identified as conjugates of ring-opened pyrazole products. The distribution of radioactive residues in tissues was extensive after seven days. In goats and hens, the sulfone was the only metabolite identified in tissues. **Plants** When applied as an incorporated soil treatment to cotton, maize, sugar beet or sunflowers, uptake of fipronil into plants in all cases was low (c. 5%). At crop maturity, the major residue components observed in all plants were fipronil, the sulfone, and the amide. Following foliar application to cotton, cabbage, rice and potatoes, at crop maturity, fipronil and the photodegradate were the major residue components. **Soil/Environment** Results of lab. and field studies: Readily degraded: major degradates in soil (aerobic) are sulfone and amide, (anaerobic) are sulfide and amide. Photolysis of soil-applied fipronil gives the photodegradate together with sulfone and amide. Koc 427 (Speyer 2.2) to 1248 (sandy loam). Both fresh and aged column leaching studies (5 soils) indicate that fipronil and its metabolites present a low risk of downward movement in soil; this is supported by field dissipation studies. Following soil incorporated in-furrow granular applications, quantifiable residues were confined to the top 30 cm of soil, with no significant lateral movement or residues.

	Soil degradation (days) (aerobic) DT_{50} (typical) 142 days, (lab at 20°C) 142days, (field) 65 days
Imidacloprid	**Pesticide type:** Insecticide, Veterinary substance **Substance group:** Neonicotinoid **Substance origin:** Synthetic **Mode of action:** Systemic with contact and stomach action. Acetylcholine receptor (nAChR) agonist. **Mol. wt.** 255.7 **M.f.** $C_9H_{10}ClN_5O_2$ **Form** Colourless crystals, with a weak characteristic odour. **M.p.** 144 °C **V.p.** 4 x 10^{-7} mPa (20 °C); 9 x 10^{-7} mPa (25 °C) **KOW** logP = 0.57 (21 °C) **Henry** 2 x 10^{-10} Pa m^3 mol^{-1} (20 °C, calc.) **S.g./density** 1.54 (23 °C) **Solubility** In water 0.61 g/l (20 °C). In dichloromethane 55, isopropanol 1.2, toluene 0.68, n-hexane <0.1 (all in g/l, 20 °C). **Stability** Stable to hydrolysis at pH 5-11. **Animals** After oral administration of methylene-14C- and 4,5-imidazolidine-14C-labelled imidacloprid to rats, the radioactivity was quickly and almost completely absorbed from the gastro-intestinal tract and quickly eliminated (96% within 48 hours, mainly via the urine). Only c. 15% was eliminated as unchanged parent compound; the most important metabolic steps were hydroxylation at the imidazolidine ring, hydrolysis to 6-chloronicotinic acid, loss of the nitro group with formation of the guanidine and conjugation of the 6-chloronicotinic acid with glycine. All metabolites found in the edible organs and tissues of farm animals contained the 6-chloronicotinic acid moiety. Imidacloprid is also quickly largely eliminated from hens and goats. **Plants** Metabolism was investigated on rice (after soil treatment), maize (seed treatment), potatoes (granule or spray application), aubergines (granules) and tomatoes (spray treatment). In all cases, imidacloprid is metabolised by loss of the nitro group, hydroxylation at the imidazolidine ring, hydrolysis to 6-chloronicotinic acid and formation of conjugates; all metabolites contained the 6-chloropyridinylmethylene moiety. **Soil/Environment** In lab. studies, the most important metabolic steps were oxidation at the imidazolidine ring, reduction or loss of the nitro group, hydrolysis to 6-chloronicotinic acid and mineralisation; these processes were strongly accelerated by vegetation. Imidacloprid shows a medium adsorption to soil. Column leaching tests (with prior ageing) with a.i. and various formulations showed that imidacloprid and soil metabolites are to be classified as immobile; leaching into deeper soil layers is not to be expected if imidacloprid is used as recommended. Stable to hydrolysis under sterile conditions (under exclusion of light). Environmental DT_{50} c. 4 h (calc., based on tests of direct photolysis in aqueous solutions). Besides sunlight, the microbial activity of a water/sediment system is an important factor for the degradation of imidacloprid. **Soil degradation (days) (aerobic)** DT_{50} (typical) 191 days, (lab at 20°C) 187days, (field) 174 days
Thiamethoxam	**Pesticide type:** Insecticide **Substance group:** Neonicotinoid **Substance origin:** Synthetic

	Mode of action: Broad spectrum, systemic with contact and stomach action. Acetylcholine receptor (nAChR) agonist. **Mol. wt.** 291.7 **M.f.** $C_8H_{10}ClN_5O_3S$ **Form** Crystalline powder. **M.p.** 139.1 °C **V.p.** 6.6 x 10^{-6} mPa (25 °C) **KOW** logP = -0.13 (25 °C) **Henry** 4.70 x 10^{-10} Pa m^3 mol^{-1} (calc.) **S.g./density** 1.57 **Solubility** In water 4.1 g/l (25 °C). In organic solvents at 20°C 1mg l^{-1} hexane, 680 mg l^{-1} toluene, 48000 mg l^{-1} acetone, 7000 mg l^{-1} ethyl acetate **Stability** pH sensitive: stable pH 1 to pH 7, DT_{50} 11.5 days at pH 9, all at 20 °C **Animals** Quickly and completely absorbed, rapidly distributed in the body and rapidly eliminated. The toxicokinetics and metabolism are not influenced by the route of administration, the dose level, pre-treatment, the site of label or the sex of animals. The major metabolic pathways are essentially the same in rats as in mice, goats and hens. **Plants** Degradation/metabolism has been studied in 6 different crops with soil, foliar and seed treatment application. The qualitative metabolic pattern was similar for all types of applications and for all studied crops. **Soil/Environment** Soil DT_{50} (median) 51 d. Stable in water under acid conditions, hydrolysed under alkaline conditions. Aqueous photolysis occurs rapidly. No bioaccumulation. **Soil degradation (days) (aerobic)** DT_{50} (typical) 50 days, (lab at 20°C) 121 days, (field) 39 days
Clothianidin (Footprint database)	**Pesticide type:** Insecticide, Metabolite **Substance group:** Neonicotinoid **Substance origin:** Synthetic **Mode of action:** Translaminar and root systemic activity. Acetylcholine receptor (nAChR) agonist. **Mol. wt.** 249.7 **M.f.** $C_6H_8ClN_5O_2S$ **Form** Colourless powder **M.p** 176.8 °C **V.p.** 2.8 X 10^{-08} mPa (25°C) **KOW** Log P 0.905 (pH 7, 20°C) **Henry** 2.9 X 10^{-11} Pa m^3 mol^{-1} (25 °C) **S.g./density** 1.61 **Solubility** In water at 20°C 340 mg l^{-1} (pH 10); In organic solvents at 20°C 1.04 mg l^{-1} heptane, 12.8 mg l^{-1} xylene, 15200 mg l^{-1} acetone, 2030 mg l^{-1} ethyl acetate **Soil degradation (days) (aerobic)** DT_{50} (typical) 545 days, (lab at 20°C) 545 days, (field) 121.2 days **Stability** Stable pH 4 to pH 9 at 20 °C, hydrolysis occurs in alkali media at elevated temperatures e.g. DT_{50} 14.4 days at pH 9, 50 °C
Acetamiprid	**Pesticide type:** Insecticide **Substance group:** Neonicotinoid **Substance origin:** Synthetic **Mode of action:** Systemic with translaminar activity having both contact and stomach action. Acetylcholine receptor (nAChR) agonist.

	Mol. wt. 222.7 **M.f.** $C_{10}H_{11}ClN_4$ **Form** Colourless crystals. **M.p.** 98.9 °C **V.p.** <1 x 10^{-3} mPa (25 °C) **KOW** logP = 0.80 (25 °C) **Henry** <5.3 x 10^{-8} Pa m^3 mol^{-1} (calc.) **S.g./density** 1.330 (20 °C) **Solubility** In water 4250 mg/l (25 °C). Soluble in acetone, methanol, ethanol, dichloromethane, chloroform, acetonitrile and tetrahydrofuran. **Stability** Stable in buffered solutions at pH 4, 5, 7. Degraded slowly at pH 9 and 45 °C. Stable under sunlight. **pKa** 0.7, v. weak base **Plants** Slowly degraded on or in plants, forming five identified metabolites (H. Saito et al., Proc. 9th IUPAC Int. Congr. Pestic. Chem., London, 1998, 2, 5A-010). **Soil/Environment** DT_{50} in clay loam 1 d; in light clay 1-2 d. DT_{50} for total residues 15-30 d. **Soil degradation (days) (aerobic)** DT_{50} (typical) 3 days, (lab at 20°C) 2.6 days, (field) 3 days
Thiacloprid	**Pesticide type:** Insecticide, Molluscicide **Substance group:** Neonicotinoid **Substance origin:** Synthetic **Mode of action:** Contact and stomach action with some systemic properties. Acetylcholine receptor (nAChR) agonist. **Mol. wt.** 252.7 **M.f.** $C_{10}H_9ClN_4S$ **Form** Yellowish powder **M.p.** 136 °C **V.p.** 3 x 10^{-7} mPa (20 °C) **KOW** Log P = 1.26 (pH 7, 20 °C) **Henry** 5.00 X 10^{-10} Pa m^3 mol^{-1} (25°C) **S.g./density** 1.46 **Solubility** In water 185 mg/l (20 °C). In organic solvents at 20°C 100 mg l^{-1} n-hexane, 300 mg l^{-1} xylene, 64000 mg l^{-1} acetone, 9400 mg l^{-1} ethyl acetate **Stability** Stable pH 5 to pH 9 **Soil/Environment** Soil DT_{50} (6 soils) 7-21 d; soil mobility (6 soils) low to medium. **Soil degradation (days) (aerobic)** DT_{50} (typical) 15.5 days, (lab at 20°C) 1.3 days, (field) 18 days

Annex 2. (Eco-)Toxicity data
Database Lab Phytopharmacy UGent

Active substance	ADI * (mg /kg BW/day)	ARfD ** (mg/kg BW)	AOEL*** (mg/kg BW/day)	MAC **** (mg/l)
Fipronil	0.0002	0.009	0.0035	0.0015
Imidacloprid	0.06	0.08	0.08	0.18
Thiamethoxam	0.026	0.5	0.08	0.018
Clothianidin	0.1	0.1	0.1	0.012
Acetamiprid	0.07	0.1	0.124 short term 0.07 long term	0.5
Thiacloprid	0.01	0.03	0.02	0.302

*Acceptable Daily Intake (ADI, mg/kg BW/day)
**Acute Reference Dose (ARfD, mg/kg BW)
***Acceptable Operator Exposure Level (AOEL, mg/kg BW/day)
**** Maximum Allowable Concentration in water (MAC). Toxicity on aquatic life (the lower the value, the higher concern); MAC = minimum ($0.01 \times LC_{50,FISH}$; $0.01 \times EC_{50,\ DAPHNIA}$; $0.1 \times NOEC_{ALGAE}$)

Annex 3. Active Substance, Product Name, Professional (P) or Private use (G), Authorisation

FPS Health, Food Chain Safety and Environment, DG Animals and Plants

	Active Substance	Product Name	Auth. Nr.	Auth. Recall.
1	ACETAMIPRID	ANTILOPSG	9845P/B	N
2		BELROSE COMBI RTU	10001G/B	N
3		DUO-STICK	9678G/B	N
4		EXXODUSSG	9898P/B	N
5		FOR-INSECT	9893G/B	N
6		FOR·INSECT RTU	9890G/B	N
7		GAZELLE	9374P/B	N
8		GAZELLESG	9807P/B	N
9		MOSPILAN	9375P/B	N
10		MOSPILAN SG	10105P/B	N
11		MULTISECT	9663G/B	N
12		MULTISECT AEROSOL	9666G/B	N
13		MULTISECT GEBRUIKSKLAAR PREP	9665G/B	N
14		ROSECLEAR	9843G/B	N
15		ROSECLEAR SPRAY	9844G/B	N
16		SUBSTRAL PLANTEN SPRAY	9667G/B	N
17	CLOTHIANIDIN	ARGENTO	9855P/B	N
18		JANUS	9499P/B	N
19		PONCHO 600 FS	9472P/B	N
20		PONCHO BETA	9474P/B	N
21		PONCHOMAIS	9823P/B	Y
22	FIPRONIL	KB MIEREN SG	9322GIB	Y
23		MUNDIAL	9196P/B	N
24		PRE MIS OMEGA	9115P/B	Y
25		REGENT FS	9197P/B	Y
26		REGENT PLUS	8941P/B	Y
27		VASCO	9297P/B	Y
28	IMIDACLOPRID	AVEVE BODEMINSECTEN GAZON	10129G/B	Y
29		BAYGON SPRAY TGN INSECTEN OP SIERPLANTEN	9139P/B	Y
30		BAZOOKA	9592P/B	N
31		BELEM	9518P/B	N
32		COMPO PLANT SPRAY	9228P/B	Y
33		CONFIDOR 200 00	9658P/B	N
34		CONFIDOR 200 SL	8686P/B	N
35		GARDIFLOR ANTI-BLADLUIS	9224G/B	Y
36		GARDIFLOR DUO PIN	9227G/B	Y
37		GARDIFLOR PLANT SPRAY	9213G/8	Y
38		GAUCH070WS	8330P/B	N
39		GAUCHO BLE	9043P/B	Y
40		GAUCHO ORGE	8955P/B	Y
41		GAUCHO R 70 WS	8396P/B	Y

42		IMPRIMO	9363P/B	N
43		KOHINOR 200 SL	9583P/B	N
44		MERIT TURF	10145P/B	N
45		MONTUR 190 FS	9234P/B	Y
46		MONTUR F.ORTE	9615P/B	N
47		NUPRID70WS	9761P/B	N
48		PROVADO COMBI PIN	8967G/B	Y
49		PROVADO GARDEN	8966G/B	Y
50		PROVADO GARDEN GAZON/INSECT	10128G/B	Y
51		PROVADO MULTICARE	9697G/B	Y
52		PROVADO PLUS	8988G/B	Y
53		PROVADO ULTRA	9466G/B	Y
54		SOMBRERO	9757PIB	N
55		WARRANT 200 SL	9527P/B	N
56		WARRANT 700	10222P/B	N
57	THIACLOPRID	BISCAYA 24000	9545P/B	N
58		CALYPSO	9352P/B	N
59		CAL YPSO GARDEN	10070G/B	N
60		CALYPSO SPRAY	10033G/B	N
61	THIAMETHOXAM	ACTARA	9916P/B	N
62		AXORIS QUICK-GRAN	9689G/B	Y
63		AXORIS QUICK-SPRAY	9660G/B	Y
64		AXORIS QUICK-STICKS	9690G/B	Y
65		AXORIS TRIPLE	9876G/B	Y
66		CRUISER	9335P/B	N
67		CRUISER 350 FS	9713P/B	Y
68		CRUISER 600 FS	9763P/B	N
69		CRUISER 70 WS	9295P/B	Y
70		CRUISER XXX	9746P/B	Y

Annex 4. Sale and use figures of fipronil and the neonicotinoids. Data are available for Belgium and Flanders

FPS Health, Food Chain Safety and Environment; Department of Agriculture and Fisheries of the Flemish public authority; Phytofar; Région Wallonne

Active substance	Belgian sales figures (kg)						
	2008	2009	2010	2011	2012	2013	2014
Fipronil	3575.05	0.00	0.00	0.00	0.00	0.00	0.00
Imidacloprid	29394.59	32825.74	28877.56	28055.96	25481.28	28760.00	23614.03
Thiamethoxam	3220.27	6304.27	9270.02	7804.12	25273.00	14704.00	7288.07
Clothianidin	1032.00	3167.60	4933.60	6187.20	7257.60	7782.00	6220.80
Acetamiprid	235.60	220.23	117.29	975.72	746.70	873.00	1611.61
Thiacloprid	5423.28	4559.76	4726.80	7189.44	5566.83	4376.00	5088.07
Total Nnc	42880.79	47077.6	47925.27	50212.44	64325.41	56495.00	43822.58

The 2014 data are not yet consolidated, and at this stage to be considered as provisional.

Active substance	Belgian use figures (kg)		
	2010	2011	2012
Fipronil	779.18	822.28	721.99
Imidacloprid	3874.74	3089.59	3115.87
Thiamethoxam	311.65	2412.55	2746.01
Clothianidin	0	0	0
Acetamiprid	214.92	797.6	702.55
Thiacloprid	4411.22	4010.39	3045.21

Active substance	Walloon use figures (kg)*		
	2010	2011	2012
Fipronil	19	0	0
Imidacloprid	22	21	4
Thiamethoxam	26	38	0
Chlotianidine	0	0	0
Acetamiprid	0.22	220	56
Thiacloprid	1217	1475	983

*"For a lot of active substances concerned no quantities applied on the treated seeds bought by the farmer are available. For certain active substances this is exactly the most important use."

Active substance	Flemish use figures (kg)			
	2009	2010	2011	2012
Fipronil	712.53	760.18	822.28	721.99
Imidacloprid	3326.44	3852.74	3068.59	3111.87
Thiamethoxam	294.09	285.65	2374.55	2746.01
Clothianidin	1074.18	-	-	-
Acetamiprid	263.08	214.70	577.60	646.55
Thiacloprid	2550.49	3194.22	2535.39	2062.21

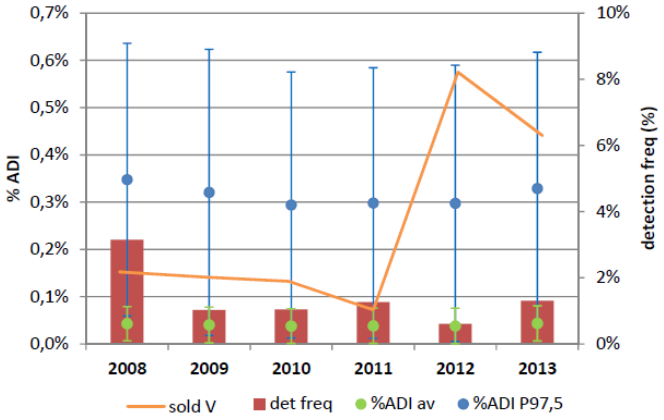
Active substance	Flemish agricultural use figures (kg)			
	2009	2010	2011	2012
Fipronil	426.32	452.72	560.60	460.04
Imidacloprid	1247.37	1426.70	1044.18	1384.91
Thiamethoxam	27.96	26.87	63.31	160.35

Clothianidin	0	-	-	-
Acetamiprid	154.45	107.82	468.98	622.53
Thiacloprid	2550.33	3194.06	2534.90	2055.15

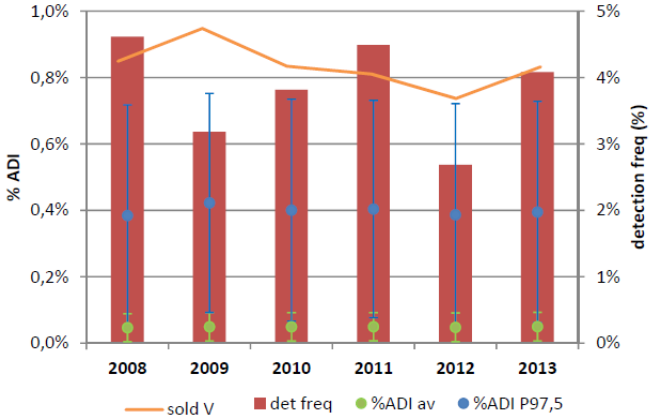
Active substance	**Flemish non-agricultural use figures (kg)**			
	2009	**2010**	**2011**	**2012**
Fipronil	0.84	0.84	0.84	1.56
Imidacloprid	273.14	506.97	311.00	222.70
Thiamethoxam	2.57	13.60	10.10	13.05
Clothianidin	-	-	-	-
Acetamiprid	285.89	281.26	285.82	63.19
Thiacloprid	0.42	0.42	1.28	18.57

Active substance	**Flemish seed treatment figures (kg)**			
	2009	**2010**	**2011**	**2012**
Fipronil	285.89	307.14	261.35	261.35
Imidacloprid	1975.28	2233.40	1906.24	1642.34
Thiamethoxam	265.16	253.62	2307.41	2580.70
Clothianidin	1074.18	-	-	-
Acetamiprid	-	-	-	-
Thiacloprid	-	-	-	-

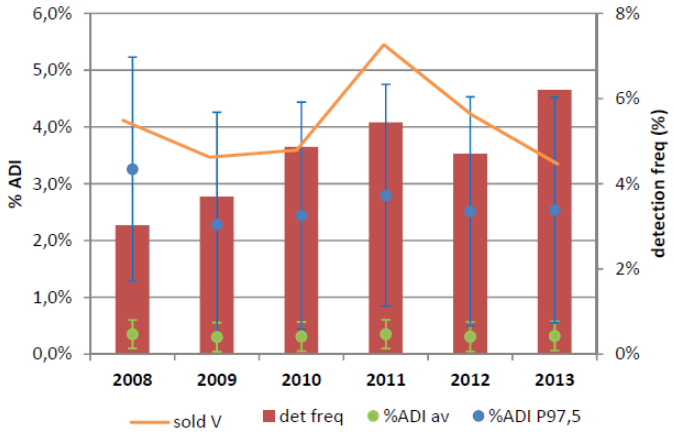
Annex 5. Advice 18-2015 of the Scientific Committee of the FASFC on the exposure of the Belgian population to residues of plant protection products between 2008 and 2013 through the consumption of fruit and vegetables



a) Acetamiprid



b) Imidacloprid



c) Thiacloprid

Quantities of pesticides (actives substances) sold in Belgium (**'sold V' : relative units), frequency of detection of pesticides residues in fruits and vegetables on the Belgian market ('det freq', %), and average and P97.5 chronical exposure, expressed as a percentage of the ADI ('%ADI av' and '%ADI P97,5', respectively) of the Belgian population to pesticides residues through fruit and vegetables consumption (deterministic approach, middle bound scenario (i.e. results < LOQ =** ½ LOQ), the higher limits of the error bars correspond to the upper bound scenario (results < LOQ = LOQ) and the lower limits of the error bars correspond to the lower bound scenario (results < LOQ = 0)). ADI are from the EU pesticide database (http://ec.europa.eu/sanco_pesticides/public/index.cfm?event=activesubstance.selection&a=1) consulted on September 2014.

Annex 6: Additional information regarding the neurotoxicity of neonicotinoids
Kimura-Kuroda et al., 2012.

Experimental design and results of the Kimura-Kuroda study

The *in vitro* system consisted of primary cell cultures of rat Cerebellar Granular Cells (CGCs), isolated from post-natal day 1 (PND1) pups, that were cultured for 14 days *in vitro* (DIV). The cell proportion was CGCs (90 %), Purkinje cells (1 %) and astrocytes (5 %). In this system, CGCs expressed several types of nAChR, which were confirmed by measuring mRNA expression of these receptor subunits at 14-16 DIV.

The cultures were exposed to 0.5 to 100 µM solutions of the test substances using continuous perfusion by means of a peristaltic pump for up to 10'. A subset of perfusions was followed up by exposure to 100 mM KCl about 500" thereafter, in an attempt to stimulate the neurons. Another subset included prior application of selective antagonists for different types of nAChRs. The parameter studied was the Ca^{2+} influx measured (indicating the neuronal excitation by the neonicotinoid agonists via the nAChR's channels) using a Fluo-4-based assay and the related excitatory patterns in cell cultures and single cells. The peak intracellular concentrations of Ca^{2+} and the proportion of the excited neurons were measured. The influence of prior administration of the antagonists was also assessed.

Following observations were reported in the Kimura-Kuroda study:

- The cells in culture were identified as >90 % cerebellar granule cells (CGC), 1 % Purkinje neurons and 5 % astrocytes, on morphological and immunohistochemical basis. The nAChR were successfully characterised by RT-PCR, while mRNA of the receptors were not expressed in non-competent renal fibroblasts.
- Administration of nicotine, ACE and IMI induced a characteristic excitatory pattern of intracellular Ca^{2+} influx at 1–100 mM in small neurons. The kinetics (Intensity *vs.* time) exhibited a representative firing pattern, *i.e*. a rapid rise and fall of signal in these cells following applications of nicotine at and a rapid rise but gradual fall in the firing patterns of these cells following applications of ACE and IMI (except ACE at 1 µM which has about the same profile as nicotine).
- Treatment with either nicotine, IMI or ACE suppressed the response of cells to an increase in extracellular 100 mM KCl (causes membrane depolarisation and thus an increase in Ca^{2+} influx via voltage-dependent calcium channels). The lack of response to KCl (100 mM) was observed after application of ACE or IMI (even after washing the compounds out), indicating that neurons were in a non-conducting, inactivated state, possibly demonstrating damage caused by these compounds to the neurons, which might not be able to respond correctly to a physiological stimulus.
  A normal Ca^{2+} influx response following KCl was observed for nicotine at the highest dose tested (100 µM), while neither 10 nor 1 µM produced this effect. The reason of this different response between top-dose and lower dose nicotine remained unexplained.
- When ≤0.5µM of nicotine, ACE and IMI was applied to the cerebellar cells, the authors did not observe significant Ca^{2+} influx during at least 3 independent replications, indicating an apparent threshold effect. It was unfortunate that intermediate concentrations had not been tested.
- It was noted that neither Purkinje cells nor astrocytes did exhibit meaningful Ca^{2+} influxes.
- At concentrations of 1 mM and above, ACE and IMI caused distinctive excitations in numerous small neurons, and the peak relative fluorescence intensities of Ca^{2+} influx did not exhibit a clear dose-dependency, but were at approximately the same level. Administration of nicotine evoked a *ca.* 1.7× higher peak of Ca^{2+} influx ($p<0.05$) than those of ACE and IMI, which exhibited similar peak values (no statistically significant differences between a.s.).

- The proportions of the neurons excited by nicotine were higher than those excited by IMI. At 1 mM, ACE excited a similar proportion of the neurons to nicotine, and both ACE and IMI at 10 or 100 mM excited similar proportions of the neurons.
- Pre-incubation with nAChR antagonists significantly inhibited the excitations and Ca^{2+} influxes in small neurons induced by nicotine, ACE or IMI at 100 mM. After removal of the antagonists, the same neurons were again excitable by the agonists. It was observed that the heteromeric antagonist also blocked the α7 response, which was unexpected and was hardly explained (potential combined responses between heteromeric and homomeric nAChR?).

A number of comments on the methodology of the Kimura-Kuroda study were identified:

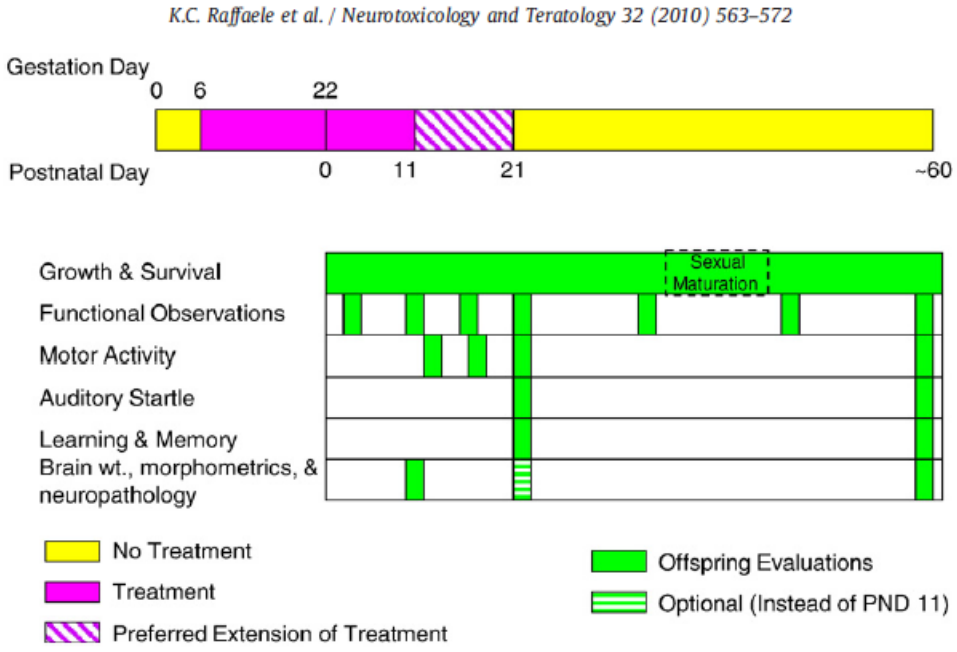
- Exposure was performed acutely at **14 DIV**, corresponding to a time of **advanced maturation** (could be considered adult neurotoxicity rather than developmental neurotoxicity).
- **Additional DNT endpoints** should have been studied to determine if key developmental processes, such as neuronal migration, differentiation, glial proliferation and maturation, might be affected by the exposure during early stages of cell culture, prior to the time when neurons are fully differentiated (approximately 10 DIV).
- The replacement at 2 DIV by serum-free synthetic medium to prevent growth of astrocytes, resulted in *ca.* 5 % astrocytes instead of 18 % (~13 % astrocytes and ~5 % microglia). Since the presence of glia (especially astrocytes) **protects neurons** against toxic insults, low content of glial cells could have less direct relevance *in vivo* (but constitutes a worst-case situation for the reviewer).
- It is not known whether the use of KCl-free medium is optimal for CGCs as it could affect the process of neuronal maturation as well as the response of the CGCs to the applied treatments (an average of 25 mM KCl would be critical for the **maintenance of the granule cells**).
- Kinetic studies of nAChR expression should have been conducted, which would have indicated **at what developmental stage these receptors are expressed**, to define what role they play in neuronal differentiation.
- nAChR antagonists were used to ascertain that Ca^{2+} influx was due to activation of nAChRs. Two antagonists believed to be selective for a particular receptor subtype were used: α-bungarotoxin for the α7 subtype (homomer, particularly abundant in developing rodent brains) and dihydro-ß-erythroidine (DHbE) for α4ß2 and α3ß4 subtypes.
- Since the **metabolic activity** of the cultured neuronal and glial cells is low or absent, the obtained results could underestimate (*e.g.* desnitro imidacloprid is more toxic than the parent) or overestimate (*e.g.* acetamiprid metabolites seem less toxic) the situation *in-vivo*.
- Minor observations pertains the exactness of the determination of the final concentrations, the low number of tested concentrations, and slight variability in the time of addition of 100 µM KCl.
- The imaging of Ca^{2+} influx was considered a useful indirect measure of action-potential generation within individual neurons, allowing monitoring the activity of a large population of neurons **at single-cell resolution**.

Regulatory studies on acetampirid and imidacloprid: methodology

In the PPR opinion, the toxicological endpoints of acetamiprid and imidacloprid are cited and discussed. For the purpose of this review, only the key studies pertaining on developmental neurotoxicity (DNT) are described in short.

Following scheme depicts the conduct of DNT assays in the EU/EPA context:
EU Test method B.53 Developmental neurotoxicity study (Reg No (EU) no 900/2014, cfr OECD TG 443)

PS: In the EU Test method B.53, the a.s. is administered from the time of implantation (GD6) until and including postnatal day 21 (PND21).



. DNT study design. A schematic representation of the exposure period and the parameters evaluated in the EPA guideline DNT study.

Additional information: Further evaluations of other neonicotinoids

- For **clothianidin**, tested at **0, 12.9, 42.9 and 142** mg/kg BW/d, there was a proper DNT which was evaluated during the EU-Peer Review (2003). Altered acoustic startle habituation, motor activity, surface righting reflex and histomorphometric findings (a.o. reduced dentate *gyrus*, *cerebellum* germinal layer, *caudate putamen*) were reported at the top-dose; the mid-dose was investigated but neither consistent behavioural adverse signs nor histomorphometric adverse findings were found. Therefore, it is considered that its most relevant DNT NOAEL (12.9 mg/kg BW/d) is covering the existing RfD.

- For **thiamethoxam**, only an EPA evaluation was available, with a DNT study where **0, 4.3, 34.5 and 298.7** mg/kg BW/d was tested. The EPA considered the a.s. devoid of any behavioural adverse effect, and overall, no consistent finding was evident. However, auditory startle reflexes could be considered altered at the top-dose. In addition, brain weights were slightly lower at the mid-dose and above. Morphometry revealed reduced thicknesses/heights and/or widths at *cerebellum*, frontal *cortex* and *thalamus* level, essentially at the top-dose. EPA noted that not all histomorphometric assessments were performed at the intermediate doses. The lowest relevant NOAEL is covering the ADI and AOEL, but is slightly lower than the ARfD. A downwards revision of the ARfD is possible, and should be verified at the occasion of a next-coming EU-evaluation.

- For **thiacloprid**, there is also a DNT study (**0, 4.4, 25.6 and 40.8** mg/kg BW/d), which is available via EPA (2003) and JMPR (2006). In the EPA-report, subtle effects on motor activity, auditory startle and passive avoidance were noted at the middle and top-dose, and thinner *corpus striatum*, *corpus callosum* and dentate *gyrus* were noted at the top-dose. The lowest dose was a NOAEL, but the EPA noted that the histomorphometric assessments were not done at the intermediate doses. However, the lowest dose could be reasonably considered sufficiently protective.

VII. COMPOSITION OF THE WORKING GROUP

The composition of the Committee and that of the Board as well as the list of experts appointed by Royal Decree are available on the following website: composition and mode of operation.

All experts joined the working group ***in a private capacity***. Their general declarations of interests as well as those of the members of the Committee and the Board can be viewed on the SHC website (site: conflicts of interest).

The following experts were involved in drawing up and endorsing this advisory report. The working group was chaired by **Luc HENS**; the scientific secretary was Marleen VAN DEN BRANDE.

ADANG Dirk	Health and environment	UCL
BOURGUIGNON Jean-Pierre	Pediatric endocrinology	ULg
DUVERGER Martine	Toxicology	WIV-ISP
GODDERIS Lode	Occupational and environmental medecine	KULeuven
HEILIER Jean-François	Toxicology	SPW
HENS Luc	Human ecology	VITO
HOLSBEEK Ludo	Risk assessment, pesticides	LNE
JACOBS Frans	Entomology, apiculture	UGent
KEUNE Hans	Ecosystem services, environment and health, risk assessment	Belgian Biodiversity Platform & INBO & UA
PASSCHIER Wim	Environmental health risk assessment	University Maastricht
SCIPPO Marie-Louise	Biocides and pesticides	ULg
SMAGGHE Guy	Biocides and pesticides	UGent
SPANOGHE Pieter	Pesticides	UGent
STEURBAUT Walter	Human exposure	UGent
VAN LAREBEKE Nicolas	Toxicology	UGent
VAN MAELE Geneviève	Pesticides and health	UCL

The advisory report has been endorsed as well by **Norbert FRAEYMAN** (Toxicology and environmental toxicology – UGent) as member of the standing working group Chemical Agents.

The following experts were heard but did not take part in endorsing the advisory report:

CASTELAIN Philippe	Toxicology	WIV-ISP
SIMON DELSO Noa	Co-author WIA-study	CARI Louvain-la-Neuve
VAN DER SLUIJS Jeroen	Co-author WIA-study	University of Bergen, Norway

The following experts peer reviewed the advisory report but did not take part in endorsing it:

NIKOLOPOULOU-STAMATI Polyxeni	Environmental Pathology, Environment and health	National and Kapodistrian University of Athens

The following administrations and/or ministerial cabinets were heard:

FONTIER Herman	Pesticides and fertilisers	FPS Health, Food Chain Safety and Environment
LAHAYE Marie-Christine	MRB Biocides	FPS Health, Food Chain Safety and Environment
VIDICK Nicolas	MRB Biocides	FPS Health, Food Chain Safety and Environment

Au sujet du Conseil Supérieur de la Santé (CSS)

Le Conseil Supérieur de la Santé est un organe d'avis fédéral dont le secrétariat est assuré par le Service Fédéral Santé publique, Sécurité de la Chaîne alimentaire et Environnement. Il a été fondé en 1849 et rend des avis scientifiques relatifs à la santé publique aux ministres de la Santé publique et de l'Environnement, à leurs administrations et à quelques agences. Ces avis sont émis sur demande ou d'initiative. Le CSS s'efforce d'indiquer aux décideurs politiques la voie à suivre en matière de santé publique sur base des connaissances scientifiques les plus récentes.

Outre son secrétariat interne composé d'environ 25 collaborateurs, le Conseil fait appel à un large réseau de plus de 500 experts (professeurs d'université, collaborateurs d'institutions scientifiques, acteurs de terrain, etc.), parmi lesquels 300 sont nommés par arrêté royal au titre d'expert du Conseil. Les experts se réunissent au sein de groupes de travail pluridisciplinaires afin d'élaborer les avis.

En tant qu'organe officiel, le Conseil Supérieur de la Santé estime fondamental de garantir la neutralité et l'impartialité des avis scientifiques qu'il délivre. A cette fin, il s'est doté d'une structure, de règles et de procédures permettant de répondre efficacement à ces besoins et ce, à chaque étape du cheminement des avis. Les étapes clé dans cette matière sont l'analyse préalable de la demande, la désignation des experts au sein des groupes de travail, l'application d'un système de gestion des conflits d'intérêts potentiels (reposant sur des déclarations d'intérêt, un examen des conflits possibles, et une Commission de Déontologie) et la validation finale des avis par le Collège (organe décisionnel du CSS, constitué de 40 membres issus du pool des experts nommés). Cet ensemble cohérent doit permettre la délivrance d'avis basés sur l'expertise scientifique la plus pointue disponible et ce, dans la plus grande impartialité possible.

Après validation par le Collège, les avis sont transmis au requérant et au ministre de la Santé publique et sont rendus publics sur le site internet (www.css-hgr.be). Un certain nombre d'entre eux sont en outre communiqués à la presse et aux groupes cibles concernés (professionnels du secteur des soins de santé, universités, monde politique, associations de consommateurs, etc.).

Si vous souhaitez rester informé des activités et publications du CSS, vous pouvez envoyer un mail à l'adresse suivante : info.hgr-css@health.belgium.be.